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MORPHOLOGICAL STUDY OF 406 CASES OF BRONCHOGENIC CARCINOMA IN JAPAN*

(With Plates XI—XVIII)

TORU MIYAJI, HAJIME KITAMURA, TSUNEAKI SENOO, TOMIO ODA and YOSHIO MURATA

(Department of Pathology, Osaka University Medical School)

A steady and remarkable increase of patients with bronchogenic carcinoma all over the world has stimulated the authors to make a survey of this disease in Japan with histologically proved cases. The present report deals with autopsy and surgically removed cases of bronchogenic carcinoma collected from universities and hospitals of almost all over the country. This is the first time in Japan that as many as 406 cases of bronchogenic carcinoma has been accumulated for pathological study.

Material and Method

18 surgical and 388 autopsy cases of bronchogenic carcinoma have been obtained by courtesy of the departments of pathology of medical schools of Tohoku, Osaka, Tokyo Medical and Dental College, Kyushu, Tokyo, Keio, Niigata, Nagoya Municipal, Chiba, Osaka Municipal, Nagasaki, Kyoto, Nagoya, Nippon Medical College, Juntendo, Jikei-kai, Shinshu, Kanazawa, Kyoto Prefectural, Hiroshima, Wakayama, Nara, Okayama, Mie, Kurume, Gifu and Tokushima, and the Second Tokyo National Hospital, Cancer Institute, National Toneyama Hospital, the First Tokyo National Hospital, Kurashiki Central Hospital and Osaka National Hospital.

A number of specimens were taken from the lung and metastases of each case, and were routinely stained with hematoxylin-eosin, periodic acid Schiff reagent, mucicarmine, Mallory, Van Gieson and Bielschowsky's method. The autopsy records were photocopied and analysed on each case, and a summary denoting the sites of origin, extension and metastasis and other pertinent information were noted.

Occupational Risks

It should be considered that the steady increase of bronchogenic carcinoma has occurred during a period characterized by remarkable technological advances.

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* Presented at the Sixth Western Regional Conference of Japanese Pathological Society at Gifu on the 9th of October, 1954.

It is the period in which increasing numbers of workers were being exposed to a greater numbers and variety of chemical and physical agents in environment, than ever before. An extensive review on this problem has recently been published by *Hueper*.[27]

In view of these occupational risks of bronchogenic carcinoma, the occupations of the patients of the present series where obtainable, were divided as follows (Tab. 1). Although detailed description of occupation was not available, professions closely related with the risk of bronchogenic carcinoma were not found.

Table 1. Occupation of 181 patients of bronchogenic carcinoma.

Occupation	Number
Clerks and office workers	49
Farmers and agricultural laborers	34*
Merchants, shop keepers and tradesmen	18
Factory workers	11**
Sea-service men	2
Chauffeurs	3
Diver	1
Coal miners	2
Cooks	3
Without occupation	12
Housewives	36

* including three women ** including a woman

Sex and Age

According to all available reports the incidence of bronchogenic carcinoma is much greater in men than women. In the present series, sex of 268 cases was stated among 406 collected cases, and it has been divided as shown in Table 2.

Table 2. Sex and histology of 268 cases of bronchogenic carcinoma

Histology	Men	Women
Epidermoid carcinoma	81	10
Adenocarcinoma	65	21
Undifferentiated carcinoma	77	14
Total	223	45

The sex ratio was found to be of the order of 5 to 1, and is close to that of the United States and England, although the incidence of bronchogenic carcinoma is much lower (3.7 males and 1.3 females among 100,000 population respectively in 1949) in Japan. It is possible that the factor mainly responsible for bronchogenic carcinoma is one to which men are particularly exposed and that the extent of

the relative difference in exposure of men is higher than women.

In the present series, 293 of the 406 cases were accompanied by data on sex and age. The youngest patient was 9-year-old and the oldest 77-year-old, and 251 patients (85%) were between the ages of 41 to 70 years, and 187 patients (64%) between 51 to 70, as shown in Table 3. It also shows 5 cases of broncho-

Table 3. Age incidence of bronchogenic carcinoma and its histology in the various decades

Histology \ Age	11–20	21–30	31–40	41–50	51–60	61–70	71–80	Total
Epidermoid ca.		2		24	41	27	9	103
Adenocarcinoma	3	3	10	20	30	18	4	88
Undiff. ca.	2	2	6	20	37	31	4	102
Total	5	7	16	64	108	76	17	293

genic carcinoma occurred under the age of twenty. No particular relation was noted between histology and age in the present study.

Initial Symptom

As the initial symptom of bronchogenic carcinoma, cough, chest pain, sputum, dyspnea, hemoptysis, weight loss, weakness, anorexia and hoarseness have been cited in the order of frequency. Unfortunately these symptoms are not characteristic and indicative of a bronchogenic carcinoma, but are common to any lung diseases including inflammatory lesions. Furthermore, a high percentage of bronchogenic carcinoma may proceed without any chest symptoms.

In the present series, 260 cases with available clinical data have been divided according to their initial symptoms as shown in Table 4. The investigation revealed that a majority of bronchogenic carcinoma showed symptoms only after it had attained a certain size in the thoracic cavity. It is noted, however, that quite a few nervous and general symptoms are initially brought by metastasis. Furthermore, 25 cases have been discovered by mass radioscopic examination, although they were not accompanied by any clinical symptoms whatsoever. *Borrie*[6] also described eight of 200 patients who were symptomless and were first detected when mass radiography revealed an unsuspected shadow in the lung fields. According to *Aufses*[7], 37 of his 959 cases had no symptoms before a carcinoma of the lung was found accidentally. Thus, as mentioned by *Moersch et al.*,[35] bronchogenic carcinoma is often so insideous in its beginning and so rapid in its development that the earliest manifestation of its presence may be produced by extension, metastasis or mortal complication. *Nakamura et al.*[36] have also stated that most of their patients came to see the doctor when they

Table 4. Initial symptom of 260 cases of bronchogenic carcinoma

Chest symptom	Cough	132
	Chest pain	98
	Fever (subfebrile 131)	94
	Hemoptysis	67
	Sputum	36
	Hoarseness	25
	Dyspnea	24
	Dysphagia	6
Symptoms related to nervous system	Neuralgia (headache, lumbago and pain of extremities)	54
	Paralysis of extremities and hemiplegia	12
	Convulsion and unconsciousness	7
General symptom	Weakness	40
	Epigastralgia, or nausea with vomiting	19
	Loss of weight	9
	Anorexia	7
	Anemia	1

Table 5. Duration of 248 cases of bronchogenic carcinoma after initial symptoms

	Months				
	1–6	7–12	13–18	19–	Total
Epidermoid carcinoma	28	27	12	7	74
Adenocarcinoma	39	29	9	6	83
Undifferentiated ca.	53	30	4	4	91

already had advanced tumors.

As to the duration of bronchogenic carcinoma after the initial symptoms, 248 cases with available data have been divided as follows (Table 5).

As far as the duration of life is concerned, it was observed that 46 per cent of patients died within 6 months and 82 per cent within a year after onset of initial symptoms. It was also noted that undifferentiated carcinoma showed the most remarkable malignancy, and was followed by adenocarcinoma and epidermoid carcinoma in the order.

Histogenesis

As tumors were of fairly large size in our series and often accompanied with extension and metastasis, it was difficult to decide the site of origin. Hence, it was felt necessary to have a small primary growth found incidentally or as yet unnoticed by patient in order to study the histogenesis. Another method of approach was to study the histologic changes of bronchial epithelium in the lung

with tumor, which was possible in the present series, and the following results as shown in Table 6 were obtained. Particular attention has been paid to reserve cells, which are postulated as a pluripotential primitive cells capable of differentiation in various directions to produce a multiplicity of possible structure (*Fried*,[18] *Halpert*[25] and *Weller*[53]).

Table 6. Histological changes of the bronchial epithelium observed in 378 cases of bronchogenic carcinoma

Proliferation of reserve cells	24
Epidermoid metaplasia of bronchial epithelium	16
Precancerous changes	4

As will be discussed elsewhere by *Taki et al*[54]., the precancerous changes are considered to be closely related to carcinoma of the bronchus as they show atypical proliferation of epithelial cells in multiple layers with a number of mitoses or with irregularity of arrangement. Serial sections of these cases revealed no direct continuity of bronchogenic carcinoma with these changes.

Concerning the proliferation of reserve cells and epidermoid metaplasia of bronchial epithelium, *Fried*,[18] *Lindberg*,[34] *Niskanen*,[38] *Weller*,[53] *Wettekind* and *Strüder*[59] suggested close relation with bronchogenic carcinoma. However, the extensive study of bronchi as was carried out by these authors could not be performed in the present series.

Macroscopical Findings

Assuming that a bronchogenic carcinoma has arisen from the site where the largest tumor was found in the lung, the primary site of 311 cases was stated in the present series and it has been divided as shown in Table 7. These data did not show much difference from what has been reported by many authors concerning the site of the primary carcinoma in the lung. For example, *Simons*[45] reported that the right lung was affected 1,147 times, the left 992 times, and

Table 7. Site of bronchogenic carcinoma found in the lung of 311 cases

Right lung		Left lung	
Upper lobe	68	Upper lobe	67
Middle lobe	13		
Lower lobe	30	Lower lobe	27
All or two lobes	12	All lobes	7
Hilar portion	37	Hilar portion	33
Pleura	5	Pleura	4
Bifurcation	9		
Dispersed all over the both lungs	3		

Table 8. Site of bronchogenic carcinoma in the lung given by *Simons*[18], *Earle*[12] and *Aufses*[2]

	Right lung			Left lung		
Author	*Simons*	*Earle*	*Aufses*	*Simons*	*Earle*	*Aufses*
Total number	358	151	560	291	136	394
Upper lobe	169	67	236	179	70	167
Middle lobe	70	14	21			
Lower lobe	119	31	171	112	34	115
Main bronchus		39	83		32	55
Uncertain lobe			49			57
Carina		4				
Uncertain lobe		38				

both in 38 instances in a series of 2,177 cases. *Simons*[18] (649 cases), *Earle*[12] (329 cases) and *Aufses*[2] (954 cases) gave the distribution as shown in Table 8. In brief, beside the preponderance of the upper lobe, slightly more tumors were found in the right lung than in the left, presumably due to the difference of weight of the parenchyma of the right lung, which averages 20 per cent more than the left, rather than the anatomcially acknowledged direction of the right bronchus.

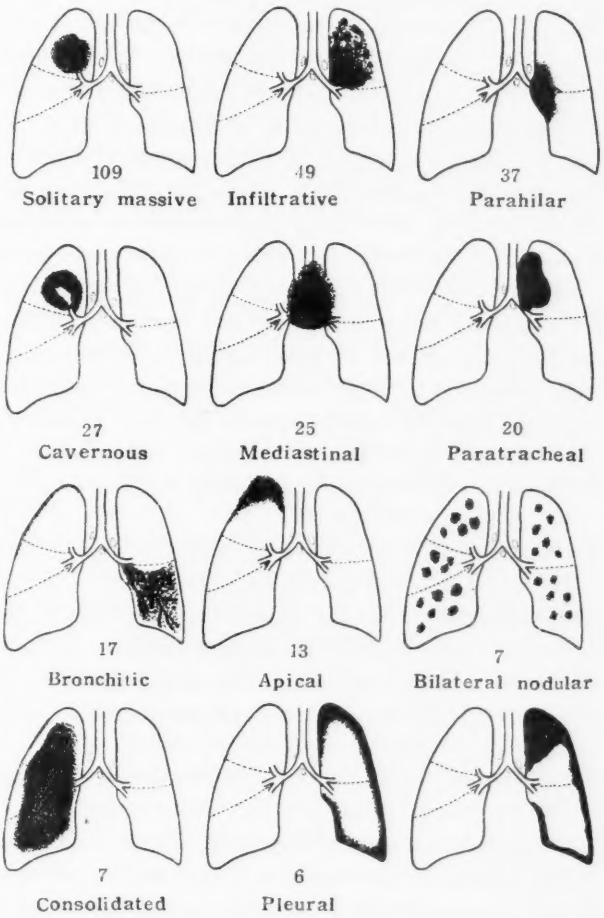
Concerning the relation between the duration of life and the site of tumor in the lung, the following result as shown in Table 9 was obtained. It also revealed undifferentiated carcinoma was more malignant than epidermoid carcinoma as far as the duration of life is concerned. The tumor located in the hilar portion seems to have longer duration compared with others. However, it may be explained on the basis that hilar tumors presented symptoms earlier than tumors of other sites.

Table 9. Site of tumor in 311 cases and average duration of life in months

Histology	Right lung				Left lung		
	Upper lobe	Middle lobe	Lower lobe	Hilus	Upper lobe	Lower lobe	Hilus
Epidermoid ca.	9.7		6.7	7.5	9.1	10.2	12.5
Adenocarcinoma	10.5	6.5	14.2	14.2	7.7	5.6	9.6
Undiff. ca.	11.0	6.6	6.7	7.1	6.4	3.5	7.9

Growth of bronchogenic carcinoma into a peripheral area or distant site can be considered to depend on the route of extension such as 1) direct, 2) through lymphatic channels, 3) blood vessels and 4) air passages, and various patterns of macroscopical picture can be observed according to the location of the primary growth in the lung. Statement of macroscopical picture was obtained in 317

Text-Figure 1. Diagrammatic outline of the bronchogenic carcinoma seen in the lung of 317 autopsied cases



cases of the present series and these cases were divided according to their appearance as show in Text-Figure 1.

In these figures, a tumor showing a mass in the lung parenchyma with pleural extension as shown in the bottom right hand was not outlined as such, and the pleural extension was not depicted. The location of tumor in the figure does not designate that the tumor was necessarily found in the exact pattern shown. The diagrams were divided according to the pattern of growth, and a tumor showing a massive growth, for example, was grouped in massive type regardless of whether it was found in the upper lobe or lower lobe.

A massive type tumor located peripherally and growing expansively with less

tendency for infiltrative growth was found in 109 cases and occupied about one third of the series. It often presented a solitary spheroid lesion in the early stage. One sixth of the tumors showed infiltrative growth in the surrounding tissue, and 37 cases were located in the hilar portion. Cavity formation in the tumor was encountered in 29 cases. A case of this group was a man of 55-year-old. His radiogram of the chest showed a lesion with horizontal line in the cavity. He was wrongly diagnosed as pulmonary gangrene, although the cavity had a thick wall with an irregular, poorly defined interior surface and a relatively well-defined external outline, which has been considered to favor the diagnosis of a cavitating neoplasm (*Moersch*, *McDonald* and *Holman*[35]). Autopsy revealed bronchogenic carcinoma with a large cavity occupying the entire lower lobe of the left lung surrounded by necrotic tissue of carcinoma. The incidence of abscess and cavity formation in necropsy series of bronchogenic carcinoma is fairly high, ranging from 12 to 29 per cent as reported by *Stang*.[51] This probably occurs when a rapidly growing tumor outstrips its blood supply, and the process of ulceration and liquefaction is aided by infarction and infection. The necrotic debris is then discharged into a bronchus, leaving an abscess cavity in the center of tumor.

13 cases of bronchogenic carcinoma were circumscribed in the apex of the upper lobe and extended to the subcutaneous tissue of the shoulder through the thorax wall with destruction of a rib. Among them, two cases presented typical "*Pancoast* syndrome" with pain in the shoulder radiating to the arm, *Horner's* syndrome and atrophy of hand muscle, which is considered to be due to a property of growth influenced by environment and not to a peculiar form of tumor (*Wurm*[69]).

A type of tumor which extended on the pleural surface without noticeable invasion or tumor formation in the lung but accompanied with a remarkable thickening of thorax wall with or without hemorrhagic effusion was found in 6 cases, and has often been diagnosed as pleural endothelioma. Several cases of the present series were diagnosed not only clinically but histologically as endothelioma or mesothelioma originating from the pleura. Careful examination and contemplation of these cases, however, revealed bronchogenic origin of the tumor in all of them except one case obtained from Nagasaki University.

Of particular interest was the tumor with multiple nodular or nodular-diffuse lesions in all lobes of both lungs. Nine cases of tumor were considered to belong to this group, and the illustrated case (Fig. 1) was obtained from Keio University which shows the typical macroscopical picture of this kind of tumor. In all of them it was impossible to determine the original site of the tumor. As they all showed a typical histological picture to be described later, they were thought to belong to the "bronchiolar carcinomas."

Histological Findings

It is well known that malignant tumor often presents quite different histological pictures, even in the same microscopical slide. Bronchogenic carcinoma is particularly notorious in this respect as will be illustrated below.

According to the classification adopted in the present study, which divides bronchogenic carcinoma into three major groups such as epidermoid carcinoma, adenocarcinoma and undifferentiated carcinoma, 383 cases, which allowed to review several portions of the same tumor, showed different types of histology as given in Table 10.

Table 10. Histological types observed in 383 cases of bronchogenic carcinoma

No. of types	No. of cases
Single type	279
Two types	102
Three types	2

These numbers revealed that in 27 per cent of the cases the morphology of tumor cells varied from area to area in the same tumor.

A surgical case from Keio University will illustrate a distinct variety of histology in the same microscopical slide as shown in the figures (Fig. 2–5). Figure 2 shows a portion of the slide with a vague suggestion of Hodgkin's disease with multinucleated giant cells. In the neighborhood (Fig. 3), more giant cells of analogous and different types were seen and remind one of the histology of pleomorphic carcinoma. This tendency is strengthened in Fig. 4, as it shows a picture of still less differentiated carcinoma. In contrast to these histological patterns, there is a histology of typical adenocarcinoma in their direct neighborhood (Fig. 5).

The histological classification of bronchogenic carcinoma has been discussed by many authors, and various points of view have been expressed. The present series was divided in three major groups as mentioned above, and these were further subdivided according to their histological characteristics and grade of differentiation. The result was shown in Table 11.

Epidermoid carcinoma: Epidermoid carcinoma of keratinized and unkeratinized type occupied the second largest number next to adenocarcinoma. Its growth was slower than the other types and had less metastasis. ***Goldman***[21] reported that epidermoid type was found in 10 out of 11 cases of bronchogenic carcinoma with a duration of more than two years. Large tumors of this type became easily necrotic in the center, and ***Strang*** and ***Simpson***[51] reported that in 44 histologically examined cases of carcinomatous abscess of the lung, 36 cases were epidermoid carcinoma.

Table 11. Histological classification of 371 cases of bronchogenic carcinoma

Epidermoid ca.	117	Keratinized	38
		Unkeratinized	45
		Under-differentiated	29
		Pleomorphic	5
Adenocarcinoma	136	Cuboidal cell	41
		Columnar cell	22
		Under-differentiated	57
		Pleomorphic	7
		Bronchiolar	9
Undifferentiated ca.	161	Oat-cell	44
		Small round cell	35
		Large cell	13
		Pleomorphic	16
		Mixed	8
Adenoma		Carcinoid	1
		Cylindroma	1

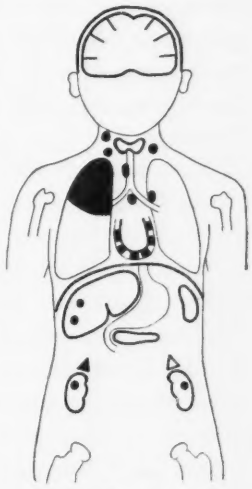
Adenocarcinoma: A typical form of adenocarcinoma is considered to present a structure with single or multiple layers of columnar or cuboidal cells standing perpendicularly on the surrounding thin or thick stroma to form a glandular space with or without mucus secretion. This kind of tumor is considered to be derived from the epithelium of a bronchus or from serous and mucous glands and their ducts.

When the typical picture of adenocarcinoma became extremely irregular, it was considerably difficult to diagnose it as an adenocarcinoma, and has been grouped in other types of tumor under various designations such as undifferentiated carcinoma, carcinoma simplex or medullary carcinoma. Meticulous examination of these cases has led the authors to believe that some of them sould be grouped as undifferentiated or underdifferentiated adenocarcinoma. A case from Tokyo University (No. 18049) was assumed to verify the authors' point. A large portion of the tumor had a histology of rather solid tumor composed of polygonal cells with nuclei rich in chromatin as shown in Fig. 6, and could be taken as an undifferentiated carcinoma, although there was an insinuation of incomplete acinar formation. A portion of this tumor had a histology as shown in Fig. 7, in which there was a manifest structure of glandular and acinar formation surrounded by a single layer of angular cells. At the same time it was also noticed that the acinar structure flowed into a solid tumor shown in Fig. 7 with vigorous proliferation of tumor cells.

The next case from Osaka University (No. 68-54) was a bronchogenic carcinoma circumscribed in the left lower lobe of the lung with continuous extension into

the left atrium protruding on its inside as a mass of 3×3.5 and 2 cm in height. There was no metastasis except in the regional lymph nodes. Most of the histology of this tumor showed fairly irregular adenocarinoma as shown in Fig. 8 accompanied with pleomorphic transformation. A portion of the tumor revealed a solid tumor with fairly large cells of clear cytoplasmic borderline suggesting the picture of epidermoid carcinoma (Fig. 9). There also was a histology which includes two pictures besides a distinct adenomatous arrangement of tumor cells. The histology illustrated in these pictures reminded the authors that all of them may be a variety of one histology named "adenocarcinoma."

The third illustrated case was obtained from Nippon Medical College (No. 13-28) and had the distribution of tumor as shown in Text-Fig. 2. Histologically, it was a solid tumor without any particular arrangement of cells in most parts. The tumor cells had vesicular nuclei of spheroid shape with translucent cytoplam stained lightly with eosin. A round nucleolus is noticed in most of cells (Fig. 10). In some areas, the same tumor cells assumed the arrangement of cylindroma intimating that the cells were derived from duct epithelium of glands (Fig. 11). A further examination revealed tumor cells of the same histological characteristics standing vertically on the stroma in single and multiple layers as shown in Fig. 12. Consequently, it seemed justifiable to assume that the solid tumor as seen in Fig. 10 was a variant of adenocarcinoma due to strong proliferation as proven by many mitotic figures, and that this histology could be grouped in an undifferentiated or under-differentiated adenocarcinoma.



Text-Figure 2. Distribution of tumor in a 56-pear-old male (Nippon Med. Coll. No. 13-28)

Another suggestion concerning the histogenesis of under-differentiated adenocarcinoma was obtained from a case of Tokyo University (No. 17489). The histology of hematoxylin-eosin stained slides as shown in Fig. 13 was not much different from the above case of Nippon Medical College. PAS stain, however, revealed scanty (Fig. 14) or abundant amount of violet-red stained thread like material, which was resistant to digestion with diastase and stained with mucicarmine. The PAS positive substance was considered to be mucus and it was found intercellularly as well as in the cytoplasm.

Of interest was a case from Tokyo Medical and Dental College (No. 324), in which clear-cut glandular arrangement and epidermoid-like structure adjacent to it were observed (Fig. 15). These findings in bronchogenic carcinoma concerning adenocarcinoma of the under-differentiated type are reminiscent of the histology of cervical carcinoma of the uterus described by *Oota* and *Tanaka*[41], who assumed

that almost one-third of their series of 56 cases of early cervical carcinoma including 26 in situ carcinomas were of columnar epithelial origin because they showed faint mucicarminophilic droplets and clear-cut transition between adenocarcinoma and solid "epidermoid carcinoma", and predominant localization of the early malignancies were found in the columnar epithelial region of the cervix. They called the solid "epidermoid carcinoma" a metaplastic metamorphosis in cancer of columnar cell origin and asserted that keratinization and spinus structures did not mean the tumor was derived from cells of squamous origin.

In the preceding discussion it was pointed out that there is a series of histologic pictures in bronchogenic carcinoma which should be grouped as an under-differentiated type of adenocarcinoma instead of being grouped simply as an undifferentiated carcinoma or carcinoma simplex. It was also considered that a bronchogenic carcinoma may have multiple sites of origin and each tumor could have different types of histology. Extensive examination of the histology of the present series, however, still revealed many cases as illustrated above and has made the authors assume that under-differentiated adenocarcinoma may present a quite remarkable variety of histology. This is one of the reasons that adenocarcinoma represented the largest number in the present classification compared with those published by many authors as illustrated in Table 12. It is noted that epidermoid carcinoma or undifferentiated carcinoma, and not adenocarcinoma was of greatest numerical importance in their classifications in contrast to the present series.

Pleomorphic adenocarcinoma: As an illustrative case of a pleomorphic adenocarcinoma, the histology of a tumor obtained from Kyushu University (No. 10028) was shown in Fig. 16. Large polygonal cells with bizarre shaped large nucleus or multiple nuclei were observed mixed with signet ring cells in the irregular glandular structure. It was the authors' impression that pleomorphic carcinoma was more frequently derived from adenocarcinoma than epidermoid carcinoma or other types of carcinoma, although some of them were classified under undifferentiated carcinoma in the present study because of the lack of glandular arrangement or stratification.

Bronchiolar carcinoma: Bronchiolar carcinoma, which is called by many synonyms such as alveolar cell tumor, alveolar cell carcinoma, pulmonary adenomatosis, diffuse carcinoma of the lung, multiple bronchiolar carcinoma and so on, was the designation when it was a tumor characterized by alveoli lined by epithelial cells of columnar or cuboidal type with preservation of the pulmonary architecture, and it was not a metastatic carcinoma from outside of the lung but was difficult to determine the original site in the lung. The above definition was given by *Swan*[53] and *Storey et al.*[50]

The histogenesis of this tumor is still obscure, but there is a trend to assume

Table 12. Histological classification of bronchogenic carcinoma by various authors

Total No.	Epidermoid ca.	Adeno ca.	Bronchiolar ca.	Undifferentiated ca.	Small cell ca.	Large cell ca.	Pleomorphic ca.	Ca. simplex	Mixed	Adenoma	Authors
47	8	5		28					6		Picco & Somaglino[13]
50	22	8			20						Beeler[1]
84	45	7	3		17	8			4	16*	Kreyberg[12]
92	49	17		26							Halpert & Pearson[21]
100	19	30	2		24	11				14	Jakobson[29]
108	35	22		42			9				Smetana, Iverson & Swan[49]
120	48	42		30							Gibson[20]
134	39	39	5		32	9					Christiansen[8]
200	111**	20		32***	37						Huguenin[23]
329	135	56		127				10			Earle[12]
331	164	66		101							Ochsner, DeCamp, DeBakey & Ray[39]
710	339	141		146	78			6		10	Aufses[2]
1000	395	137			90		378				Moersch & McDonald[38]

* 7 cases of salivary gland tumors were included.

** includes 65 cases of differentiated and 46 cases of undifferentiated carcinoma.

*** named atypical instead of undifferentiated.

this tumor originating from the epithelium of terminal bronchioles. Nine cases of this kind of tumor were obtained in the present series, and a typical case had histology as shown in Fig. 17 and 18. The architectural pattern of the lung is usually well preserved, and the alveoli were lined by tall columnar mature cells or cuboidal cells. The ovoid or elongated nuclei were located in the basal portion of cells and it was difficult to find mitotic figures. Sometimes, the alveolar wall becomes thickened with increased connective tissue and infiltration of leukocytes and round cells. Ordinarily, frothy material mixed with mucin is noticed in the alveolar spaces. As the tumor gets older, papillary protrusion and desquamated cells are found in the alveoli. There also are tumors composed of cubic cells surrounded by fairly dense connective tissue and having an appearance of adenoma. The various histology of bronchiolar carcinoma reminds the authors of the pulmonary tumor of mice produced with urethanes as shown in Fig. 19. Comparable changes have been observed in ovine and caprine infectious adenomatosis and raised the question of possible virus infection (*Swan*[53] and *Dungal*[11]).

Undifferentiated carcinoma: Histologically, undifferentiated carcinoma was defined as a tumor which shows little if any tendency or similarity to a structure or cellular arrangement observed in differentiated carcinoma such as epidermoid carcinoma or adenocarcinoma. It is more homogenous in its histology and does not change much even in metastasis.

Oat-cell carcinoma with small ovoid or short spindle shaped densely stained or vesicular nuclei with scanty cytoplasm was considered as a typical of this tumor (Fig. 20). In a few cases, large cells with large nuclei of bizarre shape or multiple nuclei were observed mixed with oat-cell carcinoma, and some of them had nuclear inclusion bodies stained with eosin. It is noted that when oat-cell carcinoma gets older the nuclei of the tumor become densely packed with chromatin and larger, and take a bizarre shape.

Undifferentiated carcinoma of small cell type is the name the present authors applied to a tumor with spheroid nuclei of the size of lymphocytes, with cytoplasm as scanty as in lymphocytes (Fig. 21). Thorough examination revealed some tumor cells with ovoid or spindle shaped nuclei, as was seen in oat-cell carcinoma, but in undifferentiated carcinoma of small cell type they were mixed in different proportions. It was speculated that there is a close relation between these two types of tumor.

The large cell type of undifferentiated carcinoma was defined according to the size of tumor cells. The illustrated case of Osaka University (No. 54-54) in Figure 22 was of a man of 32-year-old, and the tumor was originated from the upper lobe of left lung with extension downward filling up the mediastinum, invading and encircling the heart to penetrate the diaphragma to form a large mass of tumor subphrenically and in the liver. Histologically, it was a tumor

composed of large round cells as shown in Figure 22.

It was also observed that a certain type of undifferentiated tumor cells may take spindle shape under certain circumstances as shown in a case from Tokyo University (No. 18484). The primary site in the upper lobe of the right lung of a 62-year-old man showed undifferentiated carcinoma of large cell type composed of round cells with no particular structure nor arrangement (Fig. 23). Metastases were found in the brain, left supraclavicular lymph node, left lower lung, pancreas and stomach, and the metastatic nodule in the brain was partly made of spindle shaped cells as is seen in Figure 24.

Carcinomas with marked variety of size, shape and number of nuclei in tumor cells without any organized structures were included in *pleomorphic carcinoma*. The tumor cells seemed to lie close together, but each was actually separate and distinct (Fig. 25). Concerning this tumor, *Smetana et al*[49] remarked on vaguely simulated pavement epithelium without tendency towad stratification or keratinization. It was considered, however, that quite a few of these tumors were derived from adenocarcinoma as mentioned above.

Adenoma: Adenoma of the bronchus may be divided in carcinoid, cylindroma, adenoma and adenoepidermoid adenoma. A case of carcinoid and cylindroma respectively were found in the present series. Histologically, the former showed the same appearance as was observed in the intestine. Small spheroid nuclei with dense chromatin with an acidophilic cytoplasm were arranged in a trabecular pattern separated by narrow fibrous tissue bands or lined along the blood vessels (Fig. 26). Argyrophile granules were not demonstrated in this case.

In a few cases of undifferentiated carcinoma of oat-cell type, the same tumor cells with small spheroid nuclei and a narrow cytoplasmic rim were observed mixed with neoplastic oat-cells in different proportions. These observations have led the authors to consider the possibilly of malignant change of carcinoid into undifferentiated carcinoma, as mentioned by *Frey* and *Ludeke*[16], *Goldman*[22] and *Goldman* and *Conner*[23].

Cylindroma is not peculiar to the bronchus and has also been observed in tumors of the nasal and buccal cavities and pharynx. The tumor is supposed to originate from duct epithelium of serous and mucous glands, and is considered several times more malignant than carcinoid. In spite of the controversy concerning the malignancy of cylindroma (*Ritama* and *Ojala*[45]), *van Hazel et al*[56] and *Rabin et al*[44] have pointed out that cylindromas possess all the criteria of malignancy and there is no justification for comparing cylindromas with solid adenomas. *Enterline* and *Schoenberg*[13] went so far as to name it as carcinoma of cylindromatous type, as it differs in location, structure, and behavior from bronchial adenoma.

A case from Chiba University (No. 27-53) was grouped in this type as it showed

a tumor composed of vesicular nuclei with transparent cytoplasm surrounding ovoid spaces and resting on trabeculae of hyaline material in a Swiss-cheese pattern (Fig. 27). Although it had a peculiar pattern of histology, it is felt that this and other cylindromas actually are adenocarcinomas.

Relation between Undifferentiated and Epidermoid Carcinoma:

A close relationship between undifferentiated carcinoma and epidermoid carcinoma was exemplified in a case of a 56-year-old man obtained from Tokyo University (No. 18122). The tumor probably originated from the secondary bronchus of the left lung near the hilus and no metastasis was noticed extrathoracically except in the right supraclavicular lymph node. Histologically, it was an undifferentiated carcinoma of oat-cell type in the most part and an isolated area of cornification was noted in the midst of neoplastic oat-cells as shown in Fig. 28. The histological picture had a close resemblance to those described by *Barnard*[3] in 1926, who asserted that the oat-cell carcinoma, then widely accepted as a type of sarcoma, was a "medullary carcinoma" of bronchi. The same histology was also described by *Picco* and *Somaglino*[43] in 1952. Further differentiation of this kind of tumor may lead to its identification as an under-differentiated epidermoid carcinoma of the present classification. The possible connection was also noted in a case of a 50-year-old male of Chiba University (No. 157-51), and a cornification with few if any intermediate steps or stratification was noticed in a mass of under-differentiated epidermoid carcinoma. In viewing these cases, it was considered, in addition to the interpretation presented by *Barnard*,[3] that epidermoid carcinoma and undifferentiated carcinoma could have a common mother tissue, namely the pluripotential ancestor "reserve cells."

Relation between Undifferentiated Carcinoma and Adenocarcinoma:

In spite of the fact that several cases of the present series were acknowledged to prove a close relationship between undifferentiated and epidermoid carcinoma as described above, only one case merits mention in regard to the relationship between undifferentiated carcinoma and adenocarcinoma. In a case of a 64-year-old male obtained from Tohoku University (No. 48-33), undifferentiated carcinoma of oat-cell type was found histologically in most of the specimen and a small portion revealed gland formation surrounded by undifferentiated tumor cells (Fig. 29). The same interpretation may be repeated here as was expressed in the foregoing discussion concerning the probable relation of histogenesis of undifferentiated carcinoma and adenocarcinoma.

Coexistence of Bronchogenic arcinoma and Tuberculosis

Concerning the coexistence of tuberculosis in the present series, 53 cases were found to have tuberculous lesions in addtition to bronchogenic carcinoma, as shown in Table 13. As the coexistent tuberculosis was found in 53 of 406 cases of bronchogenic carcinoma, the incidence was lower than the highest figure of

50 per cent in the literature given by *Suzuki*[52] (15 cases out of 31) or 19.1 per cent reported by *Fruhling* and *Marcoux*[19] in their series of 110 cases of bronchogenic carcinoma, but higher than the average incidence of 10 per cent given by *Peters*[42] or 11.4 per cent collected from the literature by *Seyfarth*[45], and much higher than 1.8 per cent in 866 autopsy cases of bronchogenic carcinoma reported by *Bryson* and *Spencer*.[7]

Table 13. Site and nature of 53 cases of tuberculosis coexistent with 406 cases of bronchogenic carcinoma

			Character of lesion		With cavity
Same lung 29	Same lobe	18	Sclerosing	10	7
			Active	8	5
	Different lobe	11	Sclerosing	7	2
			Active	4	4
Opposite lung	16		Sclerosing	12	0
			Active	4	2
Obscure location 8					

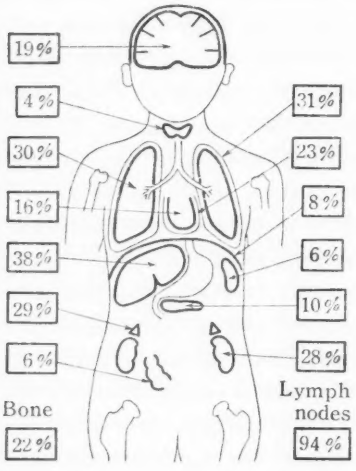
In regard to causal relation of these diseases there are many controversal opinions as represented by *Ewing*[14] who considered tuberculosis as the causal factor for the development of bronchogenic carcinoma, and by those who assumed tuberculosis merely as accidental coexistence. Concerning the latter viewpoint, *Uhlinger* and *Blangey*[55] reported there was no statistical difference in incidence of carcinoma in general among patients with and without tuberculosis. *Cooper*[9] made a comment that the association of malignant and tuberculous lesions had better be regarded as a mere coincidence of two diseases until more is known. *Cremer* and *Kaufman*[10] found 53 cases of coexistence among 350 cases of bronchogenic carcinoma and 396 cases of tuberculosis observed in 7,619 autopsies at the Frankfurt University during the five years prior to 1951. An intimate relation of each other, however, can only be postulated in the cases in which tuberculosis was found in the same lobe of lung as carcinoma. It is possible to develop a bronchogenic carcinoma in the wall of a cavity or near scar tissue of tuberculosis as cited by *Peters*[42] and *Cremer* and *Kaufmann*[10], and two cases in the present series had close relation as such. As it was recalled that very few carcinoma has been reported to develop from the wall of tuberculous cavity or bronchus among the numerous patients and autopsy cases of tuberculosis in this country, and from the fact that definitely close relation was not established in the present study except two cases and both diseases occur more often in tne upper lobe than any other lobers, it was assumed that the coexistence of tuberculosis and bronchogenic carcinoma may be merely accidental.

Of interest was the histology found in these cases. When both processes come together, tuberculous tissue is always displaced by vigorously growing carcinomatous tissue (Fig. 30). Thus, after the disappearance of caseation tissue, giant cells, monocytes and lymphocytes remained for a long time leaving the giant cells as the only rest of tuberculous lesion surrounded by carcinomatous tissue (Fig. 31). No observation has been made in the present study which would imply that carcinomatous tissue was invaded by tubercle bacilli.

Metastasis

As has been manifest from many reports, wide and early metastasis was one of characteristics of bronchogenic carcinoma. With numerical calculation of the metastasis, *Walther*[57] stressed the malignancy of bronchogenic carcinoma as worse than gastric carcinoma. According to *Fried*[17], the abundance of metastases in bronchogenic carcinome has been atrributed to two factors: 1. The rich network of blood and lymph vessels of the lungs. 2. The high degree of malignancy of pulmonary tumors.

Although *Fischer*[15] cited 20 per cent as an incidence of metastasis, *Knorr*[30] reported 98.3 per cent of metastases in his cases. In the present series, only two cases had no metastasis whatsoever. Generally speaking, metastasis of bronchogenic carcinoma may occur at any site and in any organ of the body, and metastatic sites with an incidence of more than 4 per cent in 300 cases, in which data on metastasis were available, were shown in Text-Figure 3.



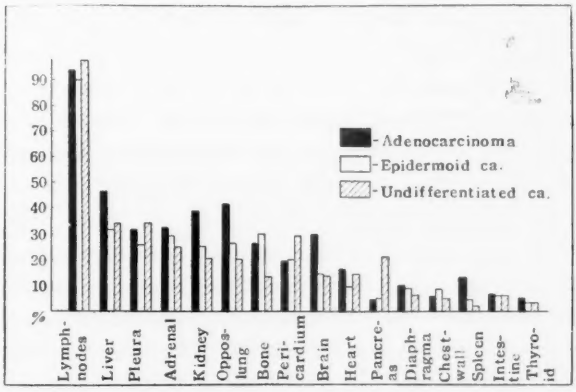
Text-Figure 3. Distribution of metastasis in 300 cases of bronchogenic carcinoma.

Metastasis was most frequently found in the lymph nodes, with the incidence of 94 per cent, and this was followed, in the order frequency, by the liver (38%), pleura (30%), opposite lung (30%), either adrenal (29%), kidneys (28%), pericardium (23%), bone (22%), brain (19%), pancreas (10%), diaphragm (8%), thorax wall (6%), spleen (6%), intestine (6%) and thyroid (4%). In 2,579 cases collected from the literature, *Ochsner* and *DeBakey*[40] found metatases to the regional lymph nodes in 75.9 per cent; liver, 34.4; bone, 20.4; adrenals, 17.6; kidneys, 16; brain, 14.6; heart and pericardium 10.0; pancreas, 5.1. As seen here, the incidence of metastases in the present study is not much different from many reports, although there is slight fluctuation in certain organs. For instance, the incidence of bone metastasis is higher than

Björk's[5] 6.8 per cent in 234 cases, *Leader* and *Borgerson's*[33] 13.8 per cent of 1,553, and as high as *Ochsner* and *DeBakey's* above percentage and *Hubeny* and *Mass*[26], 24 per cent of 150 cases. However, it was less than 32.5 per cent of *Abrams*[1] in his 160 cases, who made routine sections of the dorsal and lumbar spine.

As there have been few reports dealing with correlation of histology with metastasis except those of *Samson*[46], *Gibson*[20], *Smetana et al*[49] and *Koletzky*[31], metastases of 300 cases were analysed with the consideration of the histological pattern. Text-Figure 4 shows the relation between incidence of metastasis and histology of bronchogenic carcinoma, and revealed that adenocarcinoma was more prone to metastasize to liver, kidneys, opposite lung, brain, heart and spleen than other types of carcinoma. As to the ability to metastasize of adenocarcinoma, *Koletzky*[31] has pointed out that in his series of 33 cases of small cell carcinoma all had lymph node involvement; of 36 cases of squamous cell carcinoma, 66 per cent had lymph node involvement; and, of 19 cases of adenocarcinoma, 84 per cent had lymph node involvement. Extrathoracic dissemination occurred in only 35 per cent of the squamous cell carcinomas whereas this was present in 89 per cent of small cell carcinoma and 86 per cent of adenocarcinoma. The incidence of metastases to such organs as the brain, adrenals, kidneys, spleen, and bone was definitely higher in each case in the adenocarcinoma type than in the small cell carcinoma type.

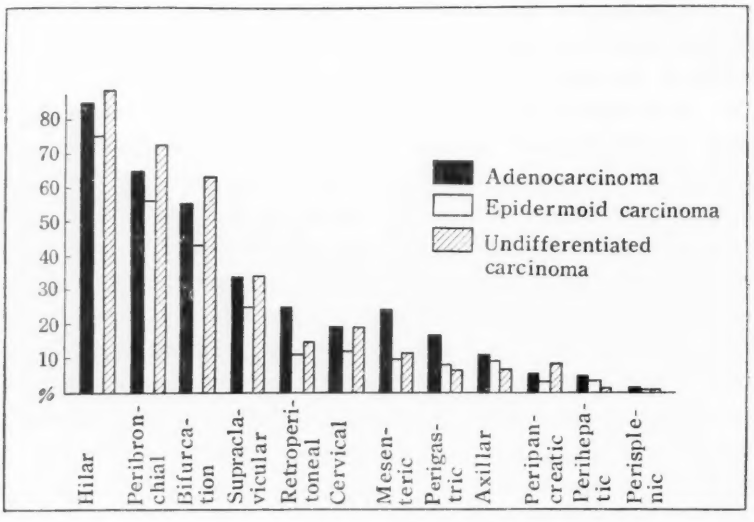
Text-Figure 4. Incidence of metastasis of 300 cases of bronchogenic carcinoma in various organs in correlation with histology



In the present study, epidermoid carcinoma had a tendency to metastasize to bone in contrast to undifferentiated carcinoma which showed affinity to the pleura, pericardium and pancreas. The same analysis has been carried out on the lymph nodes of different site as shown in Text-Figure 5. Of interest was

the high incidence of metastasis of adenocarcinoma and undifferentiated carcinoma compared with epidermoid carcinoma.

Text-Figure 5. Incidence of metastasis in the lymph nodes of various sites



SUMMARY

406 cases of bronchogenic carcinoma in Japan have been collected from medical schools and hospitals of almost all parts of the country. Although its initial symptoms and histogenesis were discussed, the principal purpose of the authors' work was to carry out a morphological study of bronchogenic carcinoma. Therefore, macroscopical classification of the tumor in eleven types is presented. Histologically, bronchogenic carcinoma was divided into three major groups of epidermoid carcinoma, adenocarcinoma and undifferentiated carcinoma, and these groups were further divided in 4 or 5 subgroups respectively according to their differentiation and histological features. Special emphasis was extended to a group tentatively named "under-differentiated adenocarcinoma", as it has a histological pattern easily confused with undifferentiated or epidermoid carcinoma.

According to the present classification, 369 cases of bronchogenic carcinoma with well preserved specimen were divided in 117 cases of epidermoid carcinoma, 136 cases of adenocarcinoma and 116 cases of undifferentiated carcinoma. As seen here, adenocarcinoma occupied the largest number of the groups in contrast to many reports dealing with histological classification by various authors, who classified epidermoid or undifferentiated carcinoma as the largest group of bronchogenic carcinoma. The difference will probably consist in the grouping of

"under-differentiated adenocarcinoma" discussed here. Two cases of bronchogenic adenomas were briefly presented, and the malignancy of the cylindromatous type is added.

Coexistent tuberculosis was found in 53 cases of 406, and its relation to carcinoma was discussed with the presentation of histology.

Concerning metastasis, only two cases were free from it, and distributions of metastases in various organs and lymph nodes of different sites were described in correlation with histological pattern.

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Explanation of Plates XI—XVIII

Fig. 1. A case of bronchiolar carcinoma. Bilateral nodular lesions with metastases in the regional and bifurcation lymph nodes and pericardium were noted.

Fig. 2. Figs. 2 to 5 show a remarkable variety of histology found in the same slide. This is a portion of tumor showing an appearance of Hodgkin's disease. Multinucleated giant cells were noted. H-E. X 210.

Fig. 3. Numerous giant cells in the tumor reminiscent of a pleomorphic carcinoma. H-E. X 210.

Fig. 4. Less differentiated carcinoma with giant cells with bizarre shaped large nuclei. H-E. X 210.

Fig. 5. A distinct histology of adenocarcinoma found in the direct vicinity of above histological pictures. H-E. X 210.

Fig. 6. Under-differentiated adenocarcinoma (Tokyo Univ. No. 18049). H-E. X 220.

Fig. 7. A portion of under-differentiated adenocarcinoma shown in Fig. 6. Acinar formation is more conspicuous (Tokyo Univ. No. 18049)

Fig. 8. Under-differentiated adenocarcinoma with a portion of pleomorphic adenocarcinoma (Osaka Univ. No. 68-54). H-E. X 220.

Fig. 9. Under-differentiated adenocarcinoma with an appearance of epidermoid carcinoma (Osaka Univ. No. 68-54). H-E. X 220.

Fig. 10. Histological picture of solid tumor observed in a case from Nippon Medical College (No. 13-28). H-E. X 220.

Fig. 11. Cylindromatous part of the same tumor shown in Fig. 10 (Nippon Med. Coll. No. 13-28). H-E. X 230.

Fig. 12. A portion of the same tumor shown in Figs. 10 and 11 illustrating the adenomatous nature of the tumor (Nippon Med. Coll. No. 13-28). H-E. X 220.

Fig. 13. Under-differentiated adenocarcinoma. Gland formation is not apparent and tumor cells are packed (Tokyo Univ. No. 17489). H-E. X 220.

Fig. 14. The same specimen as in Fig. 13, but stained with PAS reagent. Note darkly stained mucus in the intercellular spaces (Tokyo Univ. No. 17489) X 220.

Fig. 15. Gland formation and more solid epidermoid-like structure in the same specimen, in which clear-cut adenomatous arrangement was noted. (Tokyo Med. & Dent. Coll. No. 324). H-E. X 220.

Fig. 16. Pleomorphic adenocarcinoma. Large cells with bizarre shaped large nuclei are shown mixed with signet ring cells forming incomplete glandular structure (Kyushu Univ. No. 10028). H-E. x 225.

Fig. 17. Typical low-power view of bronchiolar carcinoma. H-E. X 100.

Fig. 18. High-power view of bronchiolar carcinoma showing alvéoli lined by tall columnar mature neoplastic cells. H-E. X 390.

Fig. 19. Pulmonary tumor of the mouse produced by oral administration of urethane. H-E. X 230.

Fig. 20. Oat-cell carcinoma. Irregular arrangement of tumor cells with ovoid and spheroid nuclei, and scanty cytoplasm (Tohoku Univ. No. 39-53). H-E. X 220.

Fig. 21. Undifferentiated carcinoma of small cell type (Osaka Univ. No. 168). H-E. X 100.

Fig. 22. Undifferentiated carcinoma of large cell type (Osaka Univ. No. 54-54). H-E. X 225.

Fig. 23. Undifferentiated carcinoma of large cell type composed of round cells in the primary site of the lung (Tokyo Univ. No. 18484). H-E. X 225.

Fig. 24. Metastasis in the brain of the tumor shown in Fig. 23. Transformation into spindle-shaped cells were noted (Tokyo Univ. No. 18484). H-E. X 225.

Fig. 25. Pleomorphic carcinoma. Loosely arranged tumor cells with marked irregularity in size and shape (Keio Univ. No. 3689). H-E. X 220.

Fig. 26. Carcinoid of the bronchus. Capillaries were included in the delicate stroma. H-E. X 225.

Fig. 27. Cylindroma of the bronchus with a Swiss-cheese pattern (Chiba Univ. No. 27-53). H-E. X 210.

Fig. 28. Cells with clear nuclei of epidermoid nature were noticed in the midst of the oat-cell carcinoma (Tokyo Univ. No. 18122). H-E. X 230.

Fig. 29. Glandular structure observed in the midst of the oat-cell carcinoma (Tohoku Univ. No. 48-33). H-E. X 225.

Fig. 30. Tuberculous lesion surrounded by undifferentiated carcinoma of oat-cell type (Kyoto Univ. No. 7574). H-E. X 110.

Fig. 31. Rest of tuberculous lesion surrounded and partially displaced by undifferentiated carcinoma. Giant cell of Langhans type, epithelioid cells and lymphocytes were noticed (Kyoto Univ. No. 7574). H-E. X 110.

要　旨

日本における気管支癌 406 例の形態学的研究

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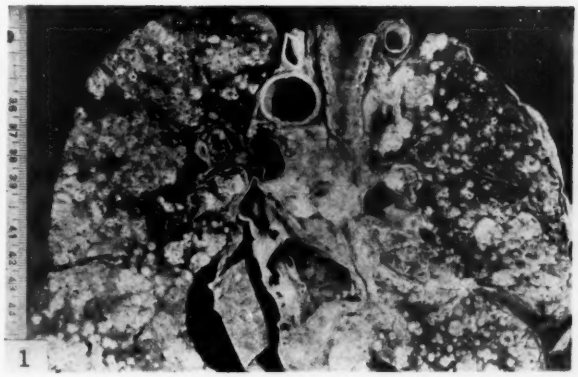
本文中に記した各大学および病院の好意によって，気管支癌の剖検例 388 と手術例 18 を集めえたので，それらについて主として形態学的研究をおこなったのが本論文である。まず肉眼的分類をおこない，ついで組織学的には，扁平上皮癌，腺癌，および未分化細胞癌に大別し，さらに腫瘍細胞の分化程度と形態を考慮してくわしく分類した。この詳細は北村によって発表されるが，仮に低分化性腺癌とよぶ腺癌がかなり多数をしめ，これは従来扁平上皮癌のあるものあるいは未分化癌にいれられていたと考えられる。したがって，ここに集めえた例のうちで充分な組織学的検査をおこないえた 369 例では，腺癌 36.9%，扁平上皮癌 32.0%，未分化癌 31.4% となり，腺癌がもっとも多数をしめている。結核との共存は 53 例にみられたが，その関係について簡単にふれておいた。転移については，くわしい研究が妹尾によって発表される予定であるが，ここでは 300 例について，組織像と転移臓器および転移リンパ節との関係をのべた。



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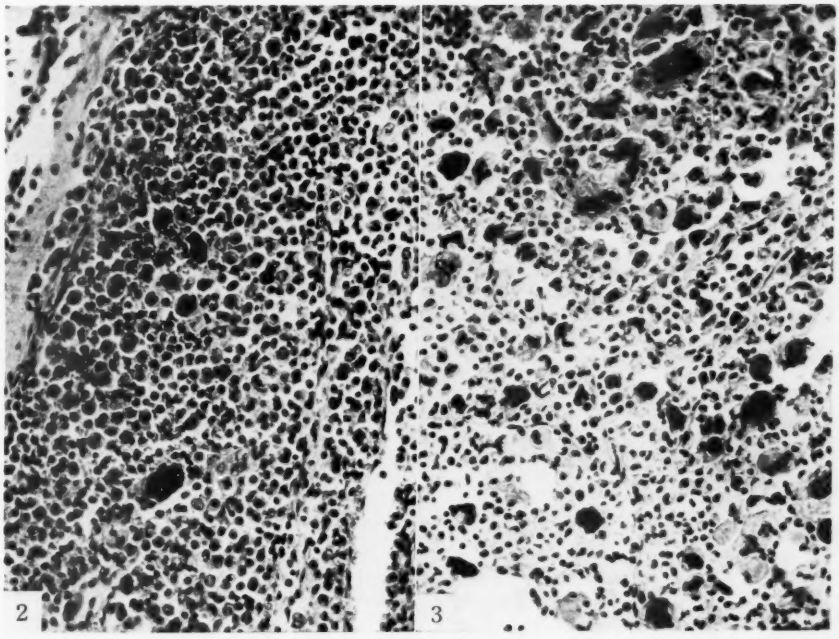

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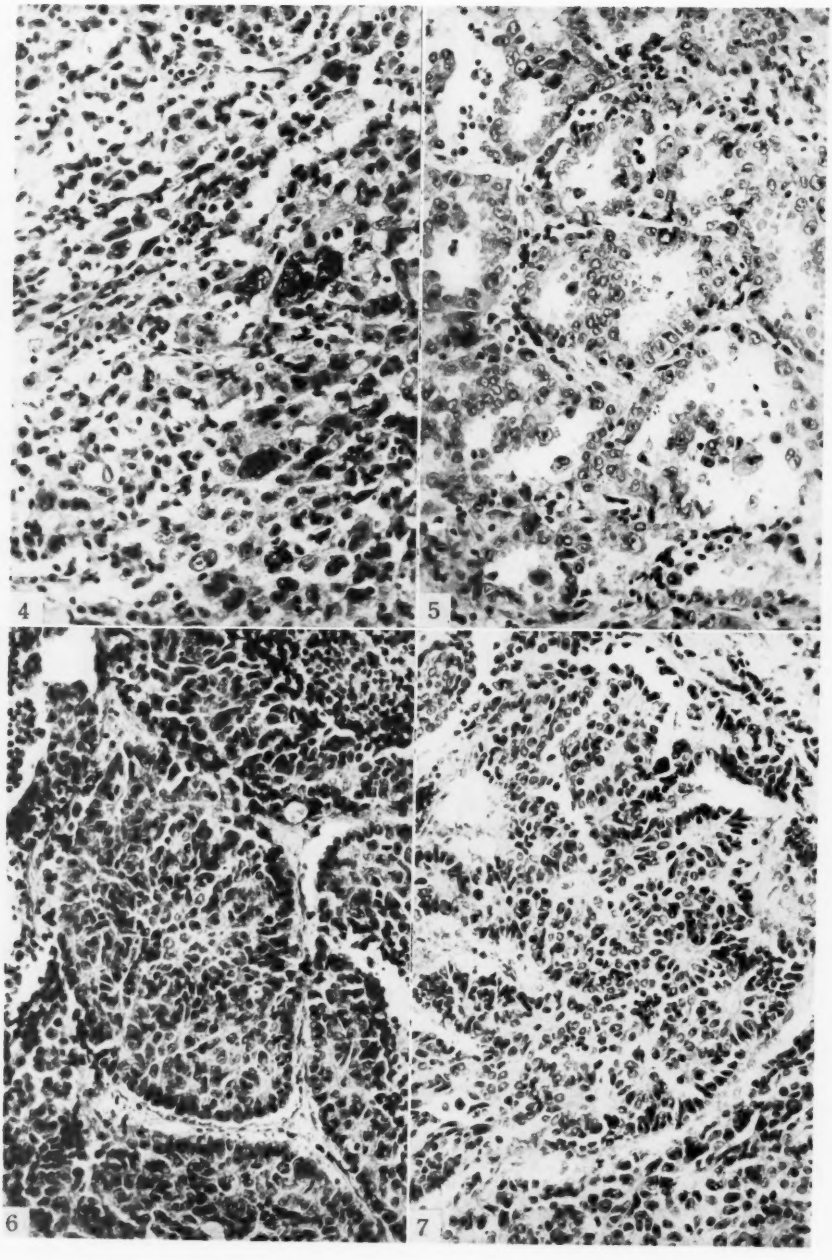
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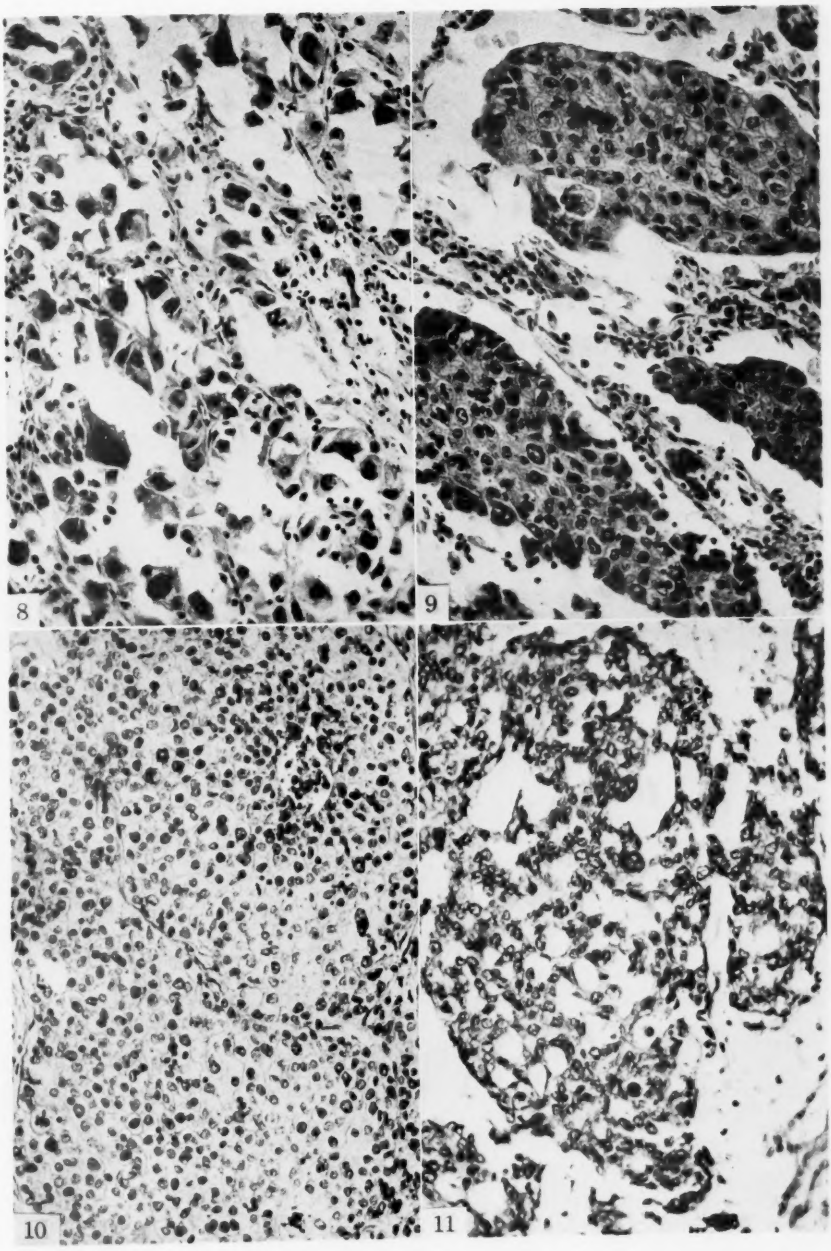
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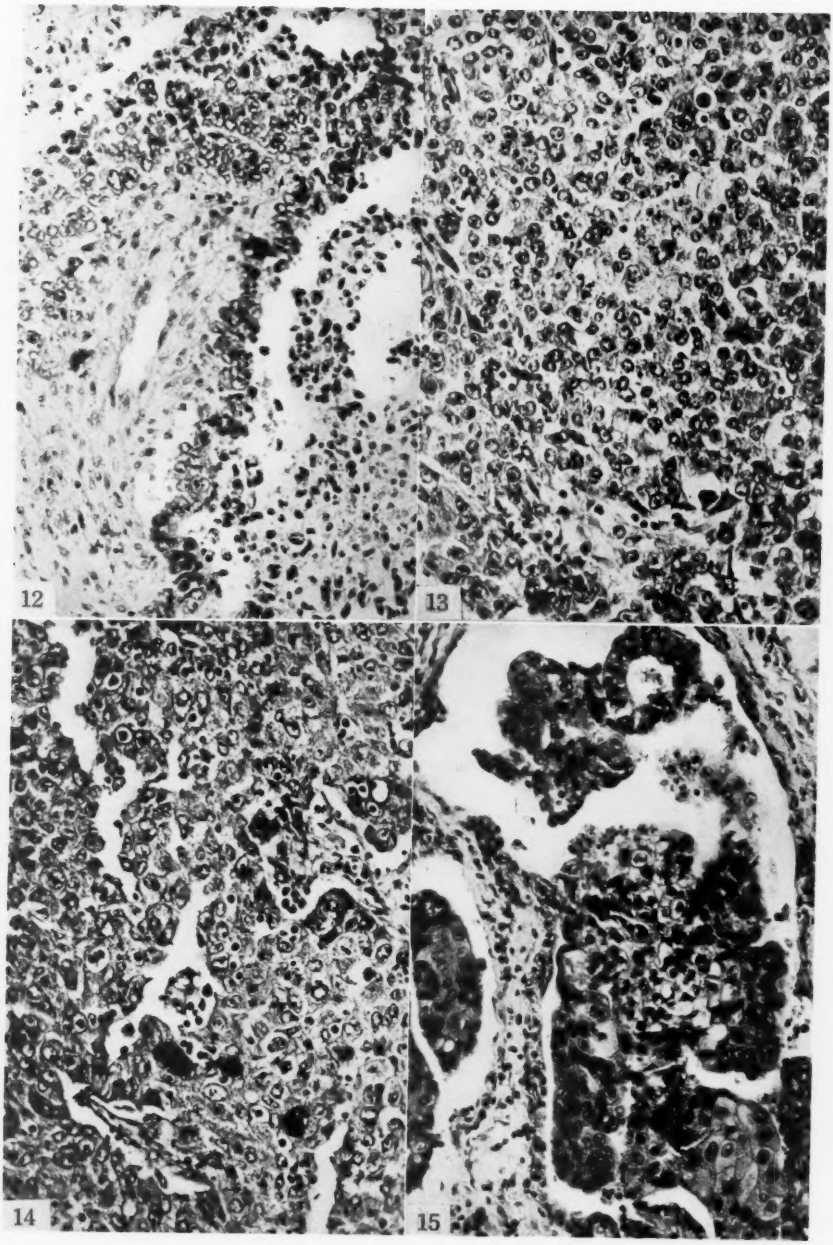
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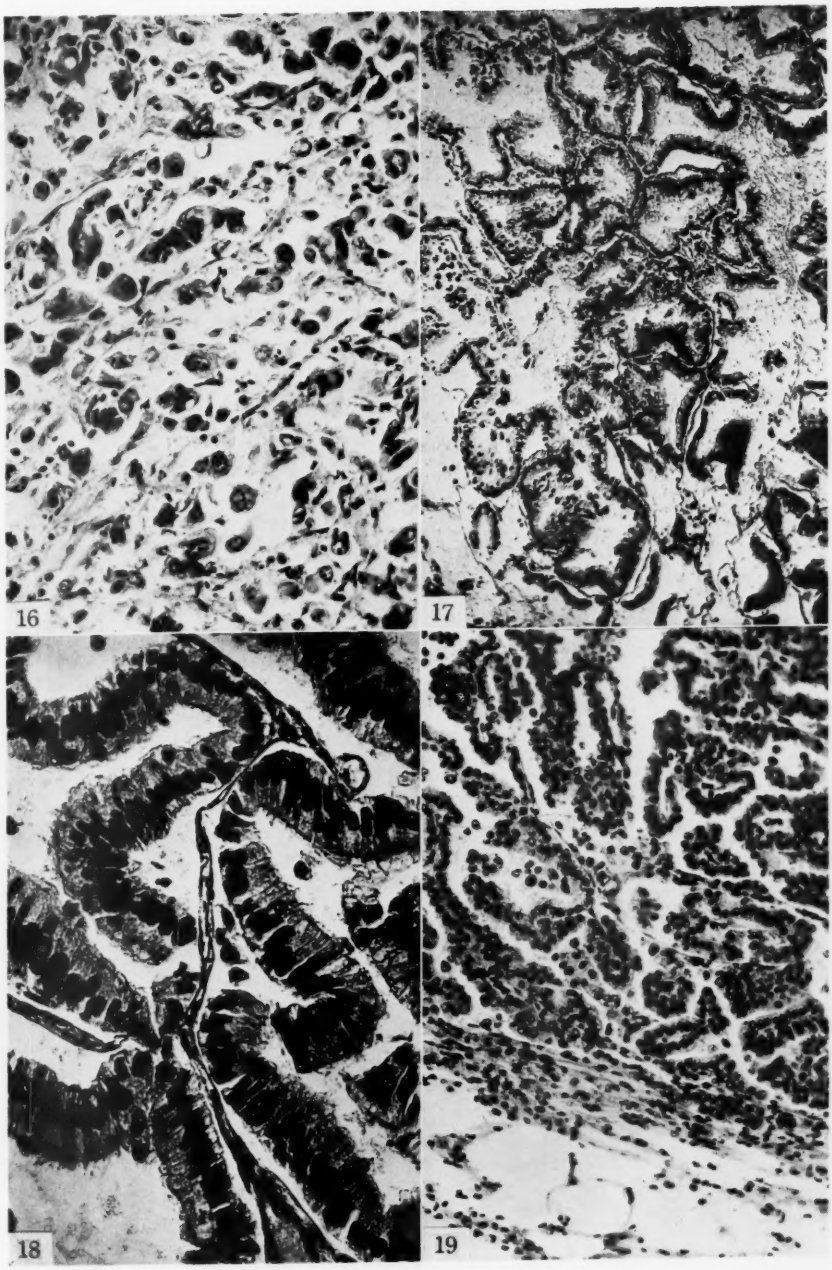
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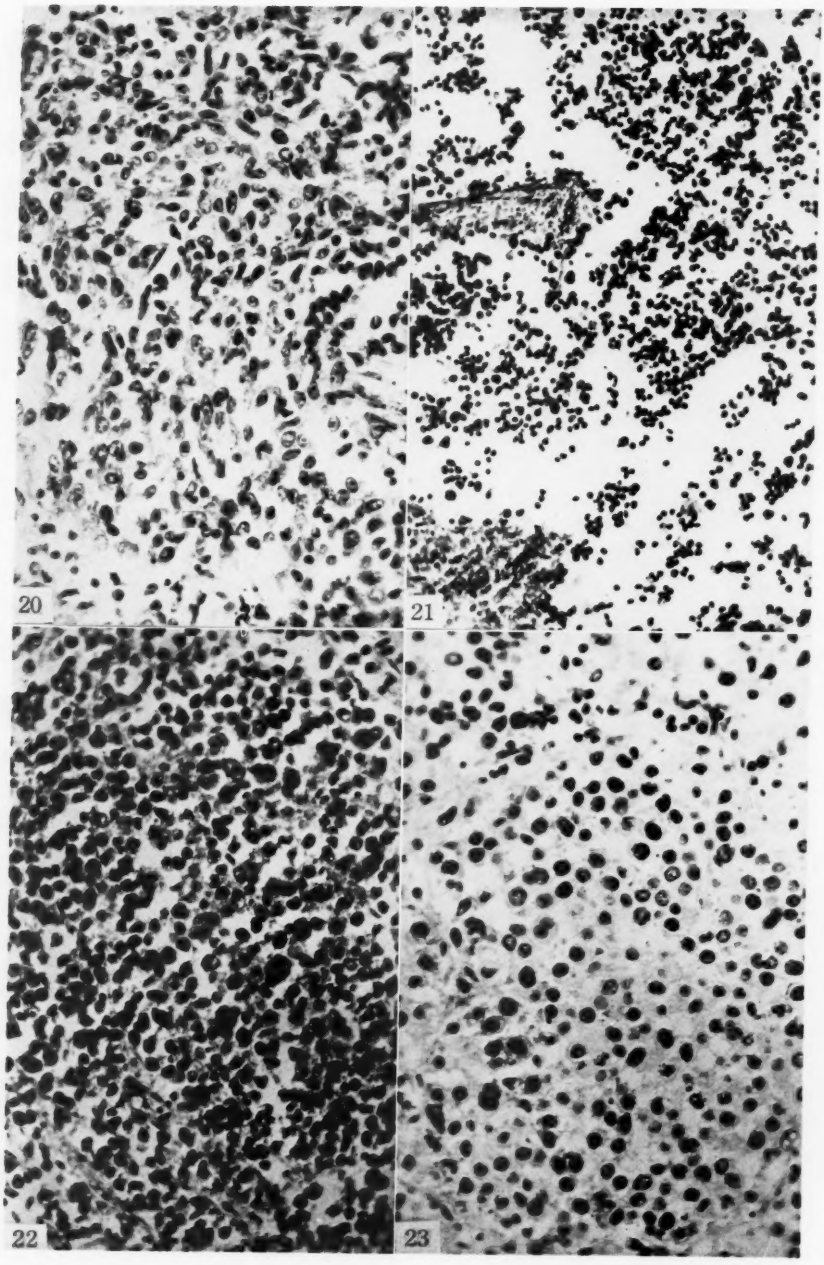
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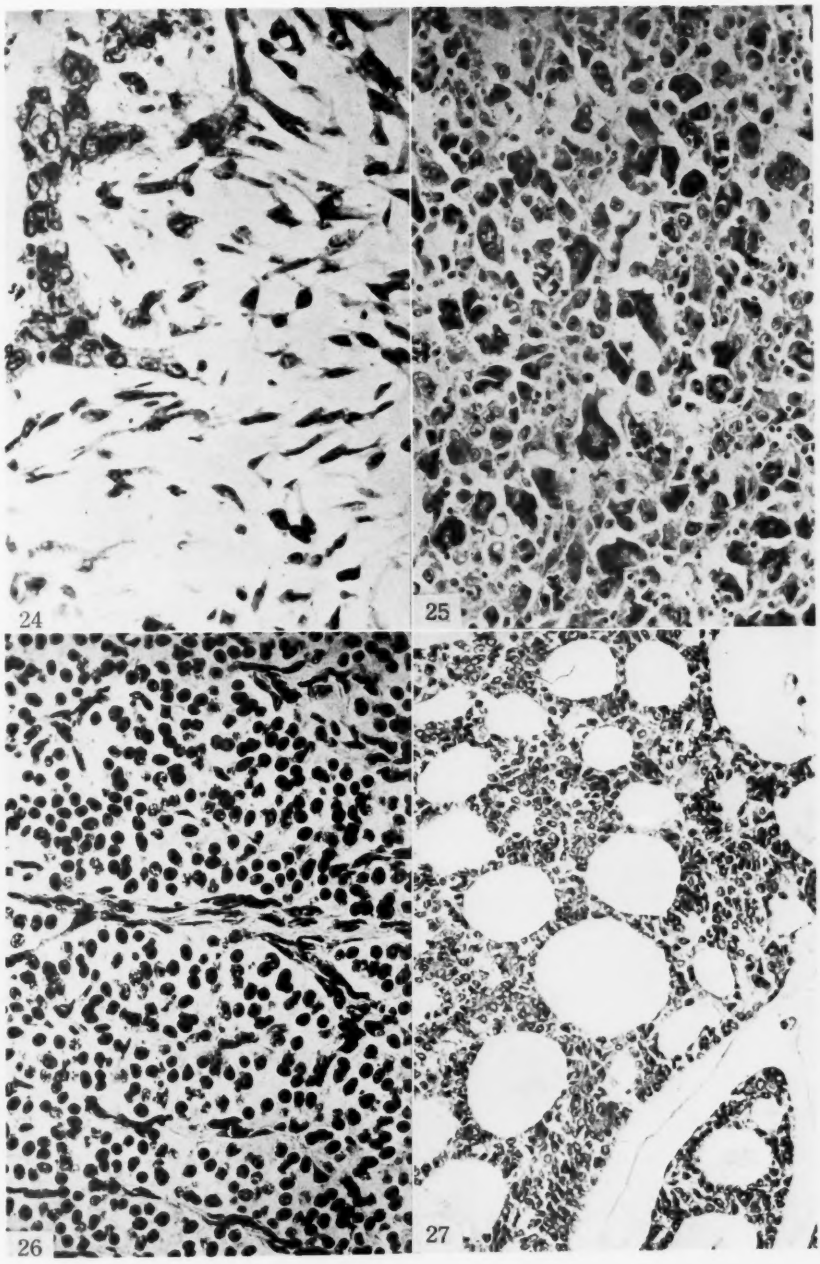
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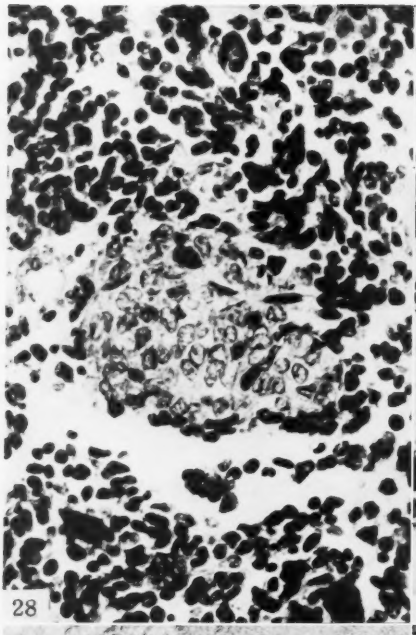
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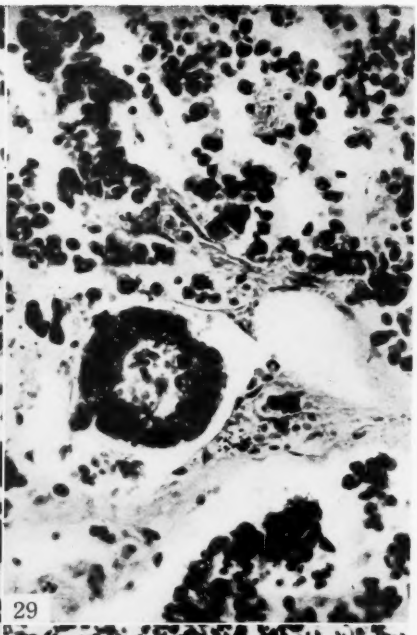
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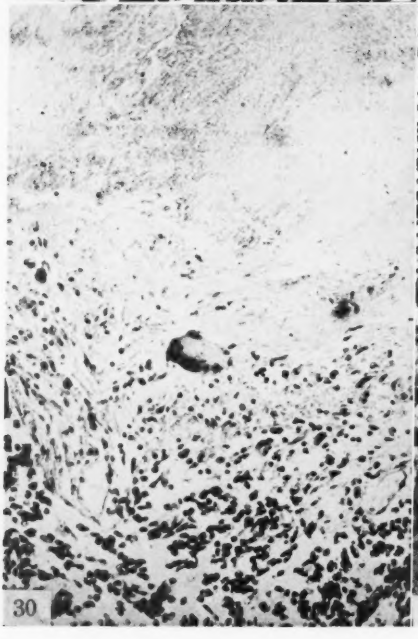
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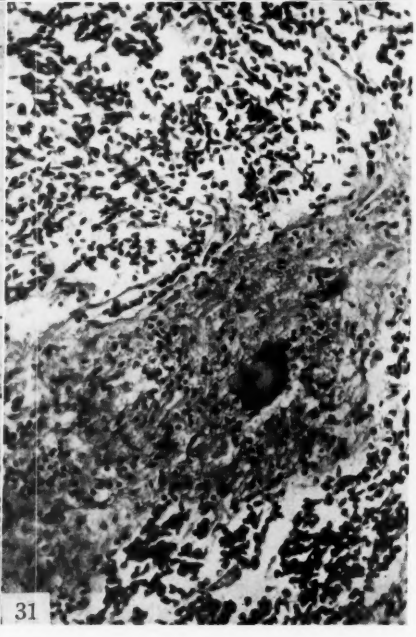
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[GANN, Vol. 46; December, 1955]

TUMOR GROWTH AND STROMAL POLYSACCHARIDES IN GASTRIC CANCER (With Plates XIX—XXII)

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Various histological patterns of tumor at the invading front into surrounding tissue are thought to be important in diagnosis of tumor malignancy. According to Willis, the infiltrative behaviors of tumor tissue are referred to autonomous proliferating and phagocytic activity, motility, production of toxic or histolytic substance of tumor cells and the correlative disorder of the surrounding tissue, and all are ascribed to the character of tumor cell itself. However, the antiblastic host reactions should be considered concurrently with tumor cell itself in analogous way to hostparasite relationship as in inflammation.

Imai, Tokoro, Okabayashi and others emphasize the importance of investigating the tumor in such a stereotypical manner. Imai, especially, studied the tissue architechtonics of cancer using a large tumor section with the whole advancing margin included in it, and pointed out the significance of peritumoral tissue, in addition to the tumor itself, as the cause of tumor "Schub" or malignant change.

Numerous reports were published on "stromal reaction of tumor," but most of the studies (Ota, Machii, Kin, Yoshida, Tanaka, Kusuhara, Muto, Miyata, Imai, etc.) are concerned with fiber architectonics of host tissue. Recently the histochemical study is in progress associated with the connective tissue chemistry, but the pathomorphological studies of stromal ground substance of tumor are relatively few, except Gersh's study of basement membrane and Chiuma's study of cutaneous cancer stroma.

The author studied the polysaccharides of stromal ground substance by various histochemical methods both qualitatively and quantitatively, using surgical and autopsy specimens of gastric cancer, and detected the correlation between the tumor growth and the stromal polysaccharide reaction and also the histogenesis of scirrhus cancer.

Material and Methods

Materials used are 5 autopsy cases of gastric cancer, and 63 surgical specimens, 2 of which are stomachs resected as a whole. Simple gastritis and ulcer as well as normal stomach were also studied for control as follows: primary stomach cancer 34, gastritis 13, stomach ulcer 15 and normal stomach 6 cases. Stomach

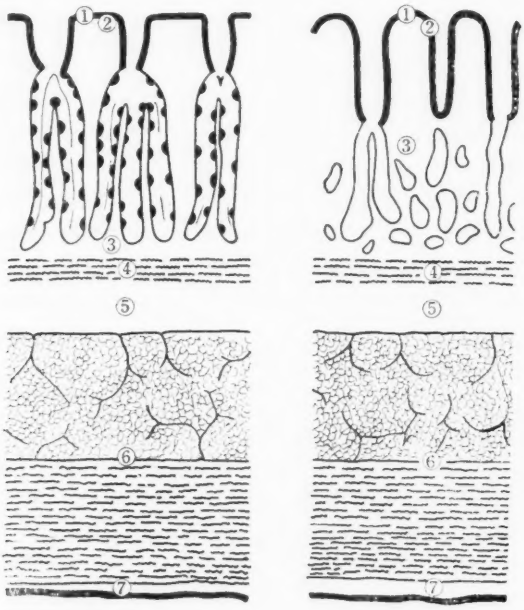
tumors such as benign tumor, squamous cell cancer or sarcoma were not examined. The specimens were fixed in 10% formol as usual, some surgical specimens were also fixed in pure alcohol.

The histochemical methods applied for demonstration of polysaccharides* are as follows: Lilie's stain* (PAS), Hale-Rinehart's* colloid iron method, Ohno's toluidin blue metachromasia,* combination of Lillie's and H–R method of Ritter-Oleson. Used also are hematoxylin-eosin stain, Van Gieson's and Azan Mallory's stain for collagenous fibers, Weigert's elastic fiber stain, and in addition, Lillie's allochrome stain and combination of PAS method and anilin blue stain after phosphomolybdic acid mordanting in order to demonstrate the relation of stromal P. and various connective tissue fibers.

The histochemically proved P. are presumed chemically to be hyalulonic acid, itin sulphuric acid, glycogen, heparin in mast cells and so on, but a part of protein molecules should also be included in the group.

The stomach wall is divided into 7 layers: 1. epithelial layer, 2. basement membrane, 3. lamina propria, 4. muscularis mucosae, 5. submucosa, 6.

Chart 1



* L.: Lillie's stain.
H–R.: Hale-Rinehart's colloid iron stain.
M.: Toluidin blue metachromasia.
PSP.: Positive substance for polysaccharide reactions.
P.: Polysaccharides.

muscle layer, 7. subserosa. (Chart 1)

The quantity of positive substance for polysaccharide reactions* (PSP) in those layers except layer 1, was estimated and expressed as (−), (±), (+), (++), (+++). In tumor case however, the tumor cell was regarded as layer 1, and the stroma at the advancing tumor margin as layer 3.

Results

1. Polysaccharide reaction positive substance in ground substance. In most specimens of formol fixation, there is inclinationally a pararellism in grade between L. and H–R. method, some portions are more reactive by the latter infrequently. The collagenous fibers themselves are stained in negative or faintly. PSP in ground substance is precipitated on the surface of collagenous and reticulum fibers, taking the appearance of agglutinated irregular mass of various sizes or interfibrilar rosarylike agglutinated droplets, the interspace of which is entirely unstained. Combination of both methods revealed that PSP above described are subdivided in alpha, beta and gamma substances as Aoki named them. Alpha substance is positive by both methods, beta and gamma are positive by either one method. PSP agglutinated on the surface of fibers is alpha in character, while the collagenous fibers themselves are beta-like in colour, but where the collagenous fibers taper off finely and P. of matrix are relatively aboundant, gamma positive coagulative substance is increased frequently in place of alpha substance.

When both reactions are applied to the specimens fixed by pure alcohol, PSP in matrix is not agglutinated on the surface of connective tissue fibers as in case of formol fixation, but stained diffusely in fine granules between fibers.

They are more intensively stained than in case of formol fixation in general, especially by H–R method and alpha substance is tinged with deeper blue, gamma substance is more or less abundant in quantity. The collagenous fibers are also reactive to some extent by either L. or H–R method, and are beta positive approximating to gamma colour tone or entirely gamma positive in nature by the combined method infrequently.

By application of toluidin blue, heparin granules of mast cells are stained red metachromatically at pH 2.5, 4.1, 7.0, while dark red coagulative mass is found interfibrilar at pH 4.1, 7.0, and the whole fiber surtace can be diffusely and faintly stained infrequently in case of formol fixation. The positive grade of this mass by this method seems, in comparison with other methods, to be parallel with the increase of gamma substance generally, but it is difficult to identity them morphologically. There is no difference in the appearance of M. depending upon pH fluctuation between the specimens of formol and pure alcohol fixation, but in the latter, the colour tone is shifted toward the short wave length principally and the latitude of positivity becomes wider, and therefore not only collagenous fiber,

cell nucleus, and mucus, but also a part of cytoplasm become clearly metachromatic. Fine granular metachromatic substances ars filled in intercellular or interfibrilar ground substance as was seen by L. method. There are further positive substances in mucous epithelial cells, duodenal excret (beta positive and no reaction by H–R), colloidal mass or blood vessel wall.

2. Quatitative estimation of stromal polysaccharides in tumor. The modes of tumor growth are classified into 4 types according to Imai as follows:

a) Hypertrophic type: Growth by the increase of thickness or volume of cancer alveoli or duct lumina.

b) Elongating type: Growth by elongation of cancer alveoli or duct lumina, the thickness of which must attain to some extent.

c) Sprouting type: Growth by shotting out as cell strands or free individual cells of cancer into surrounding tissue.

d) Intracanalicular type: Growth by permeating lymphatics or blood vessels *in loco*, especially the former.

The histological pictures of gastric cancers are classified in various aspects by many authors such as Borrmann and others, but here the classification of Imai and Tanaka is adopted, where the growth pattern of tumor and the nature of tumor cells ars arranged in good performance as follows:

1. Adenocarcinoma (A)
   - a) Microfollicular adenocarcinoma (Am)
   - b) Columnoepithelial adenocarcinoma (Ac)
   - c) Papillary adenocarcinoma (Ap)
2. Solid carcinoma (S)
   - a) Columnoepithelial solid carcinoma (Sc)
   - b) Globocellular solid carcinoma (Sg)
3. Diffuse carcinoma (D)
   - a) Globocellular diffuse carcinoma (Dg)
   - b) Mucocellular diffuse carcinoma (Dm)
4. Colloid carcinoma (C)
   - a) Adenomatous colloid carcinoma (Ga)
   - b) Solid colloid carcinoma (Gs)
   - c) Diffuse colloid carcinoma (Gd)

The number of specimens used are classified according to the above principle as follows: Am 14, Ac 11, Sg 4, Sc 2, Dg 8, Dm 3, Gs 3 cases.

1) Hypertrophic growth type (Table 1)

10 cases of this type, 12 portions of which were brought to study. 4 cases of Ac. and 1 case of G. cancer were included in them. The results in Table 1 are checked regarding 3, 5 and 6 layers (see Chart 1), the findings in the other layers were abridged for convenience. (The same rule applies to other tables that follow.)

As in Table 1, L. and H-R positive substances at the advancing margin of this growth type are insignificantly increased, poor in M. substance and collagenic fiber bundles are tortuous like thick wire with few active fibril formation. PSP are precipitated finely on the surface of collagenic fibers, and no such special

No.	Age	Sex	Hist. typ.	van Gieson			Mallory			Lillie			H-R			M.		
				③	⑤	⑥	③	⑤	⑥	③	⑤	⑥	③	⑤	⑥	③	⑤	⑥
2075	66	f	Am	++	+	+	++	+	+	++	+	+	++	+	+	+	±	±
2075	66	f	Am	++	+	+	++	+	+	+	±	±	++	±	±	++	+	±
2453	64	m	Am	++	+	±	·	·	·	++	+	±	++	+	+	++	++	±
2584	38	m	Ac	++	·	·	+	·	·	±			+			±		
2584	38	m	Ac	++	·	·	+	·	·	±			+			+		
2919	54	m	Ac	+	+	·	++	+	·	++	+		++	+		++	+	
2919	54	m	Ac	+	+	·	++	+	·	++	+		++	+		++	+	
2902	56	m	Ac	++	+	·	++	+	·	++	+		++	+		++	+	
2906	57	m	Ac	+	+	±	++	++	++	++	++	++	++	++	++	++	++	++
2184		m	Sg	++	++	+	++	++	+	±	+	±	+	±	+	±	±	±
2806	42	f	Sg	++	++	++	++	++	++	±	±	±	+	±	±	±	±	±
M. 1		f	Gs	++	++	·	++	++	·	+	±		+	++		+	++	

Table 1. Results at the advancing margin of gastric cancers. (Hypertrophic type)
③ lamina propria. (tumor stroma)
⑤ submucosa.
⑥ tunica muscularis.

localization as perivascular affinity was proved.

In case of No. 2184, the stroma at the advancing margin has a delicate capsular structure, but in spite of monocytic infiltration in tumor tissue due to bacterial infection, no extracapsular reactions take place, where the presence of PSP or M. is insignificant or failed. In 4 cases of Ac. carcinoma, the tumors are rather prone to project papillarly or cauliflower-like into stomach lumen than into submucosa or muscle wall, and there are neither increase of PSP nor proliferation

No.	Age	Sex	Hist. Classif.	van Gieson			Mallory			Lillie			H.R.			M.		
				③	⑤	⑥	③	⑤	⑥	③	⑤	⑥	③	⑤	⑥	③	⑤	⑥
2075	66	f.	Am	++	+	+	++	+	+	++	++	+	++	+	+	+	+	±
2328	68	m.	Am	++	++		++	+		+	++		+	+		+	+	
2328	68	m.	Am	++	++		++	++		+	+		++	±		++	±	
2918	53	m.	Sg	++	++	+	++	++	+	+	±	±	++	+	+	++	±	±
2869	46	m.	Ac	++	++	+	++	++	+	±	+	±	++	++	++	++	+	+
2903	60	f.	Ac	++	++		++	++		++	++					++	+	
2640	50	m.	Am	+	++	+	+	++	+	++	++	++	++	++	+	+	+	±
			Am	++	++	+	++	++	++	+	±	±	++	±	±	+	±	±

Table 2. Results at the advancing margin of gastric cancers. (Elongating type)

of collagenic fibers nor lymphocytic reaction, only mast cells migrate slightly in No. 2075 and No. 2806.

2) Elongating type (Table 2)

This type is frequently associated with the sprouting type in the studied cases, and 8 portions were selected where the former type is not so influenced by the latter. Both PSP and collagenic fibers are moderately increased in stroma.

There are 2 types of stromal collagenic fibers to be distinguished—the dense differentiated and the fine newly developed—the former arranged quite indifferent to tumor growth, whereas the latter tend to be entangled about or to envelope cancer alveoli. PSP are uniformly distributed on the surface of fine collagenic fibers adjacent to the invading front of tumor, and mottled in appearance in

No.	Age	Sex	Hist. Classif.	van Gieson			Mallory			Lillie			H.R.			M.		
				③	⑤	⑥	③	⑤	⑥	③	⑤	⑥	③	⑤	⑥	③	⑤	⑥
2156	54	m.	Am	++++	++++		++++	++++		++++	++++		++++	++++		++++	++++	
2156	54	m.	Am	++++	++++		++++	++++		++++	++++		++++			++++	±	
2183	49	f.	Dg	++++	++++	+	++++	++++	+	++++	+	±	++++	+	+	++++	+	±
2183	49	f.	Dg	++++	++++	+	++++	++++	+	+	++++	±	++++	++++	+	+	++++	+
2261	62	m.	Dg	++++	++++		++++	++++		+	+		++++	++++		++++	++++	
2289	56	f.	Am	++++	++++	+				+	+	+	++++	++++	±	++++	+	±
2408	23	f.	Sc	++++	++++	+	++++	++++	+	+	+	±	++++	++++	+	++++	+	±
2408	23	f.	Sc	++++	++++	+	++++	++++	++++	++++	+	+	++++	+	+	++++	+	+
3098			Sg	++++	++++	+	++++	++++	+	+	+	±	++++	++++	+	+	+	+
3014	54	m.	Ac	+	+	++++	+	+	+	+	±		+	+		±	±	
3014	54	m.	Ac	++++	++++	++++	++++	++++	++++	++++	++++	+	++++	++++	+	++++	+	+
2		f.	Am	++++	++++	+	++++	++++	+	+	±	±	+	±	±	±	±	±
3049	55	m.	Dg	++++	++++	++++	++++	++++	++++	++++	++++	±	++++	++++	+	++++	+	+
3047	55	m.	Dg	++++	++++	++++	++++	++++	++++	++++	+	+	++++	++++	+	++++	+	+
2669	51	m.	Dm	++++	++++	+	++++	++++	++++	+	+	±	+	+	±	+	+	+
2669	51	m.	Dm	++++	++++	++++	++++	++++	++++	+	+	±	++++	+	++++	+	+	+
2669	51	m.	Dm	++++	+	+	++++	++++	+	+	+	+	+	+	+	±	±	±
2984	40	m.	Dg	++++	+	+	++++	++++	+	+	+	±	+	+	+	+	±	±
2984	40	m.	Dg	++++	++++	++++	++++	++++	++++	±	+	+	++++	++++	++++	+	+	++++
2959	53	m.	Am	++++	++++		++++	++++		++++	++++		++++	++++		++++	+	
3082	65	f.	Am	++++	++++	++++	++++	++++	+	++++	++++	±				+	+	±
3082	65	f.	Am	++++	+	+	++++	+	+	++++	++++	±				+	±	±
2918	53	m.	Dg	++++	+	+	++++	+	+	++++	++++	±	++++	++++	±	++++	+	±
2951	54	m.	Ac	++++			++++			++++			++++			+		
3013	58	m.	Gs	++++	+	+	++++	+	+	+	+	±	+	+	+	+	+	±
3013	58	m.	Gs		++++	++++		++++	++++		+	+		+	+		+	−
2457	40	m.	Gd		++++			++++			++++			++++			+	
2457	40	m.	Dg	++++	++++	++++	++++	++++	+	+	++++	+	++++	++++	+	++++	++++	+

Table 3. Results at the advancing margin of gastric cancers. (Sprouting type)

perivascular space occasionally (No. 2869). Between the tumor tissue and the PSP area, there is always a narrow free zone. The results by L. and H–R method run in pararell principally, but in No. 2869 H–R is intensively positive in contrast to faint L., where the fine fibrils are poorly developed and the dense fibers are tumefied.

M. substace is also increased especially in No. 2869. Cellular reactions take place in moderate degree, but mast cell reaction is variable in each case and not so marked in No. 2869.

3) Sprouting type (Table 3)

In 28 portions examined, the active proliferation of collagenic fibers, increase of H–R positive substance and M. are noticed in general.

When the collagenic fibers are not so extremely developed, positive grade by L. and H–R method seems to run in parallel, while it does not where the fibers proliferate actively and densely as in case of scirrhus or carcinoma fibrosum. In such a case, H–R positive substance is often strikingly increased in contrast to L. positive substance, where the fine collagenic fibers are irregularly entangled or dense differentiated fibers are tumefied. Such loosely meshed areas are enveloped by dense proliferative zone of differentiated collagenic fibers, where L-positive substances are distributed interfascicularily in wide area. PSP is increasing in mottled appearance as in case of the elongating type and often in close contact to tumor cells or in perivascular space. L. positive and H–R positive substances are distributed in antagonistic manner in the sprouting type. The relationship between H–R positive substance and collagenic fibers as described above is however inapplicable to some cases (No. 2669), where PSP is not so increased as the fine collagenic fibers (see the lower part of Table 3, and Figs. 7 8).

As to the elastic fiber, it proliferates mainly in muscularis mucosae and outer zone of submucosal layer near the advancing tumor margin regardless of any growth type and has no relation to PSP. condition.

3. Stromal findings of inflammative, ulcerative and normal stomach wall. 6 normal, 13 inflammative and 15 ulcerative cases were brought to investigation. In normal case, collagenic fibers are few and make dense bundles. PSP in ground substance is also scanty, precipitating on fiber surface. In case of acute and chronic gastric catarrh, there is no deeper bacterial invasion and no remarkable difference compared to normal case. In case of chronic ulcer, PSP is generally increased but very variable in quantity in each case compared with the constant presence of dense fibrosis.

An old scar tissue contains only sparsely distributed PSP occasionally, which is something different from tumor case. PSP is uniformly distributed and no local difference is present in non-neoplastic cases.

4. Topographic difference of stromal polysaccharides reaction in the same

tumor. The findings above described are concerned with the relationship of PSP and collagenic fibers of stroma at the advancing front of tumors of various types. But, since the growth type of the same tumor can be variable in many places, and is surrounded by different tissue structures, topographic difference of stromal response of the same tumor should be expected.

Some representative cases are demonstrated as follows:

Case 1. Colloid carcinoma (No. 2457) (Fig. 9)

Typical colloid cancer, the external surface of which is ulcerated and invades through muscle coat up to subserosa. At the invading front in muscle layer, goblet cells are floating in mucinous mass by which the surrounding tissue is compressed. Where the muscle tissue is adjacent to mucinous mass, PSP is poorly developed and restricted to interfascicular spaces, and a few non-mucinous secreting tumor cells sprout into the surrouding tissue. The interfibrilar deposition of PSP is few and restricted to the sprouting focus of non-mucinous secreting cells. The tumor cells are encountered by active deposition of PSP at subserosa and cease to grow outward. In submucosa on the other hand, non-mucinous secreting cells sprout in strands actively toward lateral direction, where PSP and M. increase strikingly *in loco*. It is characteristic in production of fine collagenic fibers and a few mast cells are in those areas. From the fact that there is considerable difference in P. reactions in two places, muscles and submucosal layer, it is presumed that the reaction is not the expression of host body as a whole against tumor, but is influenced by local conditions. It is also noted that the mucinous substance and the cells secreting it do not stimulate production of PSP so actively as non-mucinous secreting cells, and the influence of histological and chemical characteristics of muscle and submucosal layer upon P. reactions should be taken into consideration.

Case 2. Ulcerative cancer (No. 2918) (Fig. 10)

The mucle layer covered by thin coat of scar tissue is exposed to the ulcer ground and overhang the surrounding catarrhalic mucous membrane at the right margin, from where the cancer seems to develop and spread over the ulcer surface and into submucosa permeating into lymphatics. The submucosal tumor on the right side (see the schema) develops in tubular structure (elongating type), a part of which penetrates muscularis mucosae and arrives at submucosa. At the advancing margin of tumor, the stromal H-R positive substance, M. substaece and collagenic fibers are increased in moderate degree, a few mast cells are scattered among them.

In the ulcer ground opposite to the area above mentioned, the tumor cells poor in cytoplasm are sprouting in cell strands along the cicatrified muscle bundles. Here are PSP and M. insignificant as in normal stomach, and fine collagenic fibers are poorly developed among dense fiber bundles. P. reactions are only

faintly positive on fiber surface in scar tissue free from tumor invasion. Agglutinative precipitation on the fiber surface or in interfibrous space is entirely negative. The advancing margin in this area arrives at subserosa, where the tumor cells obtain rich protoplasm and form wide alveoli, and PSP also increases correspondingly. This case indicates that the growth type and the stromal P. reactions can be influenced by the local environment.

Case 3. Globocellular diffuse carcinoma (No. 2183) (Fig. 11)

The tumor cells, cubic or round in shape, form small alveoli and invade from submucosa into muscle layer (on the left half of the schema). PSP esp. H–R positive substance and collagenic fibers are increased surrounding the tumor cells infiltrating into muscle layer. Where PSP are abundantly produced, fine reticular structures are made up by precipitated mass interfibrarly, which show up apparently by van-Gieson's and Mallory's method. There are also capillary formations in those areas, and much PSP are deposited in perivascular spaces where the relatively dense fibrous elements are loosely meshed and run independently to each other and fine fibrils are developed on the other hand. In the muscle layer (on the right half of the schema), isolated tumor cells are infiltrating interfascicularly accompanying the slightly increased PSP and poorly developed fine collagenic fibers. M. is slight in the former but moderate in muscularis in degree, mast cells are also present to some extent in the latter.

Case 4. Colloid diffuse carcinoma (No. 2669) (Fig. 12)

The tumor cells are separated from each other, distributed diffusely and uniformly in submucosa (on the left half ot the schema), which is covered by relatively intact epithelium. All tumor cells contain in their cytoplasms L. positive red mucous droplets, some of them are changing into goblet cells. In this area the collagenic fibers proliferate actively and is demarkated definitely against the loose connective tissue area free from tumor cell invasion.

No increase of PSP, M. or mast cells in the tumor area. These facts seem to have some relations to the lack of non-mucous secreting cells.

Discussion and Conclusion

Before discussing the results above obtained, some problems on the histochemical procedures for P. should be considered. The results of histochemistry for P. depend upon the fixation of specimens. Aoki divided P. into alpha, beta and gamma by using Ritter-Oleson's combined method, and thinks gamma substance, because of its water solubility, is impossible to prove histochemically by formol fixation. According to Ohneda, hyalulonic acid is prone to be transfered into water soluble fixatives, and Lison could extract it with water from the specimens fixed by acetone. He could also prove the glycogen to some extent histichemically by watery fixatives and supposed a part of glycogen combined with high molecules

could be fixed to some extent when the protein is adequately fixed. The author compared the formol and pure alcohol fixatives applied to the same specimen in regard to the positivity of P. reactions, and found PSP precipitate in agglutinated status on the fiber surface in the former, in fine granules in the latter, but quantitatively there is no difference between them.

In addition, I could not find any reduction of these substances in deparaffinized sections rinsed in water for three days. Gamma substance is also well preserved, which indicates that PSP escapes little if any, when the specimens are fixed rapidly and completely by formol. It must be considered here that the formol does not permeate into tissue so fast as alcohol. From the aspect of chemical natures of L. positive and H–R positive substances, they should be always demon-

		Gastric Cancer			Gastritis	Ulcer	Normal
		Hypertr-troghic	Elong-ating	Sprout-ing			
van Gieson	−						
	±						
	+～++	6(50.0)	2(35.0)	5(17.9)	10(71.4)	3(12.5)	4(66.7)
	++～+++	6(50.0)	5(62.5)	14(50.0)	4(28.6)	8(33.4)	2(33.3)
	+++～++++		1(12.5)	7(25.0)		11(45.8)	
	++++～			2(7.1)		2(8.3)	
Mallory	−						
	±						
	+～++	7(63.6)	2(25.0)	5(18.5)	9(79.2)	2(9.1)	2(40.0)
	++～+++	4(36.4)	5(62.5)	11(40.7)	4(20.8)	8(36.4)	3(60.0)
	+++～++++		1(12.5)	9(33.4)		9(40.9)	
	++++～			2(7.4)		3(13.6)	
Lillie	−						
	±	4(33.3)	1(12.5)	1(3.7)	3(21.4)	2(8.3)	1(16.7)
	+～++	6(50.0)	5(62.5)	7(25.9)	11(78.6)	17(70.8)	5(83.3)
	++～+++	2(16.7)	2(25.0)	18(66.7)		4(16.7)	
	+++～++++			1(3.7)			
	++++～					1(4.2)	
Hale-Rinehart	−				1(8.3)		
	±	1(8.3)			1(8.3)		2(33.3)
	+～++	8(66.7)	4(57.2)	14(53.9)	10(83.4)	14(60.9)	4(66.7)
	++～+++	2(16.7)	2(28.5)	10(38.5)		6(26.1)	
	+++～++++	1(8.3)	1(14.3)	2(7.6)		3(13.0)	
	++++～						
		12	8	28	14	24	6

Table 4 Estimation of PSP of tumor stroma. Numbers in parenthesis indicate percentage of case numbers when each method is spplied.

strated in parallel, but as the results I obtained, they are often in discrepancies, and in case of pure alcohol fixation, not only collagenic fibers become faintly positive, but also M. extends up to nucleus and cytoplasm.

From these facts, it is not deniable that histochemical reactions for P. are significantly influenced by the fixatives used and the ultimate histochemical identification of P. is indefinite. The author can not go too far into this complex but fundamental problem of histochemistry, and must be satisfied in detecting the correlation of PSP and tumor stroma histochemically by use of specimens rapidly fixed by formol.

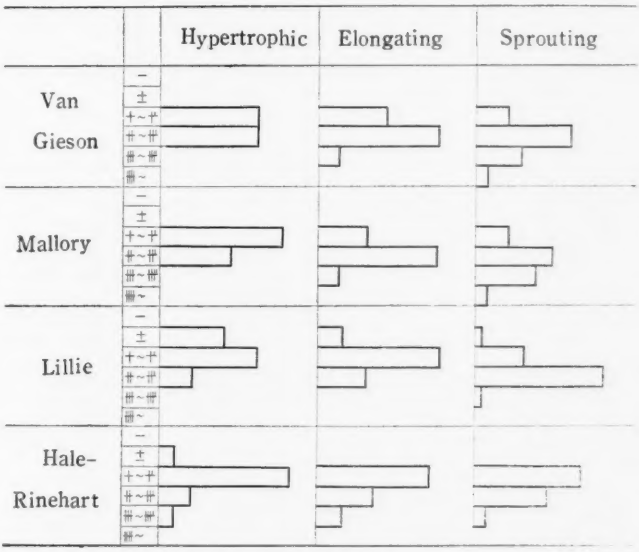
The histochemical data are arranged and summarized according to each growth type of tumor and histological picture in Tables 4 and 5 and graphically demonstrated in Chart 2. In tumor case, it is arranged in order of the sprouting, elongating and hypertrophic types in regard to the positive grade for P. reactions, the former two are approximately the same in their degrees.

The proliferation of collagenic fibers adjacent to the advancing tumor margin also runs parallel with P. reactions. In gastritis and in normal case, there is no increase of PSP in contrast to definite increase in ulcer case.

However, the inconstant presence of L. positive substance in comparison to the marked increase of collagenic fibers in ulcer case is noticed.

As regards the relation of PSP and histological pictures of cancer, Dg and Ac carcinomas are situated at both extremities as to the degree of P. reactions, other types are ranked between them. But in one and the same tumor mass,

Chart 2



there can be often found some variations or differences in growth type or histological picture topographically and also local stromal reaction correspondingly. When the results obtained from histological classification (Table 5) are compared with those from growth types (Table 4), many facts are revealed, for instance, Am carcinoma has tendency to be varied in growth type in different localities and the stromal response is dependent on each growth type, Ac carcinoma contains much PSP infrequently because of its predominance of hypertrophic type and Dg carcinoma invades tissue by sprouting mainly, as a result of which P. reactions are intensified.

Further it is presumed the stromal reactions are influenced also by the tissue conditions invaded by tumor as in case of muscle layer, ulcer ground, scar tissue, submucosa, etc.

		Am	Ac	Sg	Sc	Dg	Dm	Gs
van Gieson	−							
	±							
	+～++	1(6.7)	8(72.7)	1(25.0)		1(12.5)	1(33.3)	1(33.3)
	++～+++	11(73.3)	2(18.2)	2(50.0)	2(100.0)	4(50.0)		2(66.2)
	+++～++++	3(20.0)	1(9.1)	1(25.0)		3(37.5)	1(33.3)	
	++++～						1(33.3)	
Mallory	−							
	±						1(33.3)	
	+～++	1(9.3)	9(81.8)	1(25.0)		1(12.5)		1(33.3)
	++～+++	10(71.5)	1(9.1)	2(50.0)		4(50.0)		2(66.7)
	+++～++++	2(19.2)	1(9.1)	1(25.0)	2(100.0)	3(37.5)	2(66.7)	
	++++～							
Lillie	−							
	±		3(27.2)	2(50.0)		2(25.0)		
	+～++	12(80.0)	5(45.5)	2(50.0)	2(100.0)	3(37.5)	3(100.0)	3(100.0)
	++～+++	3(20.0)	2(18.2)			3(37.5)		
	+++～++++		1(9.1)					
	++++～							
Hale-Rinehart	−							
	±			1(25.0)				
	+～++	7(53.8)	6(63.6)	2(50.0)		3(37.5)	3(100.0)	3(100.0)
	++～+++	6(46.2)	2(18.2)	1(25.0)	1(50.0)	3(37.5)		
	+++～++++		2(18.2)		1(50.0)	1(12.5)		
	++++～					1(12.5)		
		15	11	4	2	8	3	3

Table 5. Estimation of PSP in each type of cancer. Numbers in parenthesis mean the same as in Table 4.

There are often discrepanies between stromal response and growth type especially in the former.

On the problems of stromal reaction of tumor, various interpretations were postulated but no definite conclusion at present.

It is because the reaction is influenced by many components, and a tumor as a whole is discussed. Imai demonstrated the histological difference of one and the same tumor both topographically and chronologically and could analyze the complex histology of cancer by application of prototypes of tumor growth designed by him to each advancing tumor margin.

His analytical method is also applicable to the stromal P. reactions in many aspects. From the stromal P. reactions and the fiber formation *in loco*, cancer is classified in 4 types referring to Imai's principle as follows:

1) Non-reactive type. Both PSP and collagenic fibers are poorly developed or failed. It is found in hypertrophic growth type, gastritis and a part of sprouting growth type.

2) Polysaccharide type. In this type, PSP is much increased in contrast to the poor development of collagenic fibers, which are characterized by fine fibers and often degenerating dense fibers. PSP is most reactive by H-R method, M. is most intensive in degree and mast cells emigrate actively. This type is found in stroma at the advancing front of sprouting or elongating growth type and further in pericapillary space near it. PSP is mottled in appearance and the loosely meshed fibers are stained like edematous sweeling by the ordinary connective tissue stain.

3) Fibrous type. PSP is poor, collagenic fibers are predominant as in connective tissue callus of stomach ulcer. M. substances and mast cells are absent and collagenic fibers are densely bundled.

4) Polysaccharide-fibrous type. This is transitional type from 2 to 3. PSP is abundant and uniformly distributed on surface of proliferating collagenic fibers, M. is faintly positive, mast cells are not in appearance. The invading front of elongating and sprouting type or a part of ulcer cases belong to this type.

PSP patterns of the 4 types above described are intimately related to proliferation of collagenic fibers and growth types of tumor. The relationship of polysaccharides in matrix and collagenous fibers is being elucidated recently correlating with the problem of morphogenesis of collagenic fibers.

It is not concluded at present about the origin of collagenic fibers, intra- or extracellular. Porter or Miyata proved intra-cellular profibril formation by electron microscopy, Szent Georgi found plexus of profibrils arranged on the surface of fibrocytes. Chiuma, on the other hand, studied stromal P. reactions in experimental tumors and divided them into 5 stages. He explains that P. in matrix is depolymerized in his early stages, resulting in M. and then repoly-

merized as cancer develops, collagenic fibers change in parallel with PSP.

The stromal P. is thought to be induced from blood born component (Gersh) or from intraplasmic production of fibrocyte excreted into intercellular space (Miyata).

Chiuma refers M. to the degree of polymerization of stromal P. When the author's classification above described is compared with Chiuma's stages, there can be found common chronological change of precesses of stromal reactions.

When the tumor invasion takes place, the stromal P. is increased and deposited also in perivascular spaces and the already differentiated dense fibers are degenerated, which seem to be the same phenomenon that Tokoro and Kusuhara explained as "perifokale Mesenchymverjüngung," "Blastom-bedingte Mesodermauflösung" or "Demaskierung des Kollagens".

It is reasonable to think PSP about cancer alveoli as the result of depolymerization of local P. as Chiuma concludes, but that in perivascular space distant from cancer cells is difficult to be explained as the effect of depolymerizing enzyme of cancer cells. It is rather sound to derive them from blood born components as Gersh. Miyata distinguishes between the P. produced by fibrocyte and that originated from blood components and correlates the latter to serous inflammation. But in fact, it is difficult to distinguish them, and the exudate in serous inflammation is different in histo- chemical response. They may originate from blood vessels through some different mechanism.

The fact that PSP in perivascular space often spreads to considerable extent, whereas that about cancer alveoli does not increase to such an extent, makes them mainly referable to the blood born components. The blood vessel distribution must be also taken in consideration. In some cases (Case 2), the faint stromal reaction is already described in spite of the sprouting type of tumor developed in scar tissue, it may be responsible to the deficiency of blood vessels in scar tissue and the variance of positive degree according to each layer of stomach is thought to be related to blood vessel distribution to some extent. The spreading factor in tumor tissue and antispreading factor and its inhibiting factor are also proved in tumor patients, further is reported the presence of antitrypsic substance in blood though their presence was denied by Kiriluk.

But the phenomenon of depolymaerization at the invading front of tumor, the foreignness of tumor growth, localization of P. reaction corresponding to each growth type, some unexplainable facts in case of hypertrophic type or colloid cancer, all these facts seem to indicate the importance of the substances above mentioned.

The outsanding increase of PSP in case of gastric ulcer also is full of suggestion in relation to gastric cancer from the viewpoint of histolytic enzyme. On the other hand, it is reported also the inhibiting factor in blood is P. itself, and the relation between the appearance of PSP and blood vessel distribution is of

much attractive interest.

Summary

34 cases of gastric cancer and control (gastritis, ulcer and normal stomach) were studied histochemically with regard to polysaccharides of ground substance.

1. At the advancing front of tumor, the stromal P. and the collagenic fibers are brought in intimate correlation.

In this aspect, the stromal reactions of both cancerous and non-cancerous stomach wall can be divided into 4 types as follows: a. Non-reactive type. b. Polysaccharide type. c. Polysaccharide-fibrous type. d. Fibrous type.

2. In case of gastric cancer, the hypertrophic growth type at the advancing margin (Imai) corresponds to (a), while the elongating or sprouting type (Imai) to (b) and (c). Some relationships between the growth types of cancer (Imai) and the stromal P. reactions are confirmed.

3. The stromal P. in the same tumor is influenced not only by various growth types but also by different histological pictures. PSP at the advancing front of tumor does not mean the response of the whole host body, but the mutual rela tion between the local tumor tissue and the stroma.

4. The stromal P. is produced from the surrounding of the advancing margin of tumor and the pericapillary spaces near it, P. from the former is reasonably referred to the depolymerization of ground substance, whereas blood born components can also play an important role in producing it.

5. Toluidin blue metachromasia, which is thought to indicate the grade of polymerization, comes up most outstandingly in case of polysaccharide type than other types.

6. The proliferative stimulus of producing stromal P. is most referable to the histolytic enzymes produced by tumor.

7. The stromal reactions are influenced not only by tumor tissue, but also by environmental tissue structures. The capillaries near the advancing margin seem to play a role in the reaction.

8. P. in ground substance is precipitated on surface of collagenic fibers in agglutinated appearance in case of formol fixation in contrast to alcohol fixation. PSP in ground substance does not so easily disappear from ground substance by formol fixation as is usually believed.

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Explanation of Plates XIX—XXII

Plate XIX

Fig. 1. The advancing front of hypertrophic growth (Sg) No. 2184.

Fig. 2. Colloid cancer of hypertrophic growth (Gs) M. 1.

Fig. 3. Intracanalicular growth (Am) No. 2289.

Fig. 4. Elongating growth (Am) No. 2328.

Fig. 5. Elongating growth (Am) No. 2075.

Fig. 6. Sprouting growth (Sg) No. 3098.

Fig. 7. Sprouting growth (Dm) No. 2669.

Fig. 8. Hale-Rinehart's stain of Fig. 7. Mucous secreting cells are intensively positive but PSP poor in ground substance. Fine collagenic fibers are actively developed with few cellular elements.

Plate XX

Fig. 9. Colloid cancer. No. 2456. Sprouting growth of non-mucous secreting cells in submucosa (upper left).

Fig. 10. Ulcerative cancer (Am) No. 2818. Elongating growth at the right and sprouting growth at the left. Lymphatic invasion at the right.

Plate XXI

Fig. 13—Fig. 20. Mallory's connective tissue stain, for demonstration of various proliferative

status of connective tissue in accord with tumor growth.

Fig. 13. Normal stomach wall. S 111.

Fig. 14. Gastritis acuta. No. 2366. Submucous edema and capillary proliferation. No increase of fibrous elements.

Fig. 15. Ulcer. No. 3069. Connective tissue callus formation up to subserosa. PSP also increased in this case.

Fig. 16. Ulcerative cancer No. 2918. Adenocarcinoma arising from the ulcer margin. Lymphatic invssion of tumor in the middle part (compare Fig. 10).

Fig. 17. Solid cancer of hypertrophic type. No. 2184. Thin membranous capsule formation at the advancing margin. No extra-capsular deposition of PSP.

Fig. 18. (Am) carcinoma. No. 2075. Tumor mass to the upper right side. Elongating growth with sprouting growth in part. Active connective tissue proliferation in submucosa demarkated from intact submucosa.

Fig. 19. Colloid cancer. No. 2457. See explanation of Fig. 9.

Fig. 20. (Dm) carcinoma. No. 2669. See Fig. 12. PSP is restricted to the sprouting area.

要　旨

胃癌発育時における間質多糖類の態度

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腫瘍組織の発育像はその悪性度診断の上に重要な所見とされているが，炎症における と同様 Host-parasite Relationship 追究の要請されねばならぬことはすでに諸家の触れる所である。かかる二重の生物学的見知に立ち人胃癌発育の態度とともに同所間質内多糖類の所見を組織化学的に検索を行った。

腫瘍は同一例にあっても部位環境によってその組織像並びに発育型を異にするが，間質多糖類の所見もまた腫瘍の発育環境，発育型の変化に伴って変動し，この種反応が個体の腫瘍に対する全体的表現でなく，局所的な腫瘍，間質間の相互関係に規定せられると同時にまた該部位の膠原繊維の所見と密接なる関係を持つことが認められる。その個々の部位における反応態度を同所の膠原繊維の所見と併せわれわれは次の如き四型に分ちこれを整理した。

1) 無反応型；多糖類反応陽性物質 (PSP) の増量が顕著でなく，膠原繊維もまた増生を見ない。

2) 多糖類型；膠原繊維の新生は極めて乏しく，一部に既存繊維の膨化が見られ，かつ PSP の増量顕著なるもの。

3) 多糖類繊維型；前者と次の繊維型との移行型と考えられるもので，PSP 増量とともに

微細膠原纖維の増生が見られる。

4) 纖維型； 膠原纖維の増生，生長著しく，PSP はむしろ減少している。

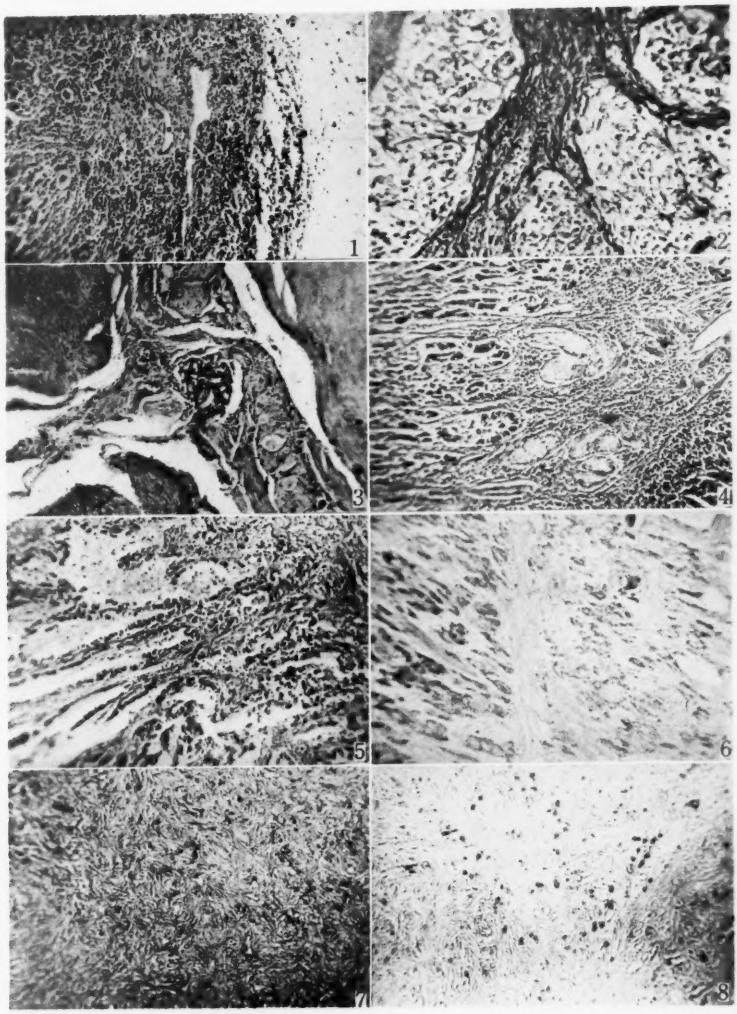
多糖類の重合度を示す異染性は多糖類型に強く，他の型に属すべき反応部にては極めて微弱であり，中馬の成績と比較考案し，これらの諸型は反応の時間的経過に因るものと考えられる。

以上の諸型を腫瘍の発育型（今井）に対応せしめると肥大発育部は無反応型を，延伸発育部および簇出発育部にてはその時間的経過に応じて多糖類型，多糖類纖維型を示し，とくに簇出発育部位ではしばしば多糖類型を示し，腫瘍の発育型と多糖類性反応の間には一連の関連を求めることができる。

これら PSP の増量は腫瘍発育先進部および近隣血管周囲より起り，前者については中馬の述べるが如き局所基質の解重合に基く PSP の増量とともに，血液成分由来の PSP の存在もまた考慮せしめられ，壊疽部，筋層における多糖類性反応の微弱なる事は同所の血管保有量の乏しい事にも依るものであろうか。

之等 PSP の増量刺戟として，腫瘍の異物性，腫瘍発育に伴う組織の離断，並びに膠様物質の化学的刺戟よりむしろ近時多数報告されつつある腫瘍組織の産生する諸組織融解性酵素 (Hyaluronidase, Trypsin 等) を重視したい。

最後に組織化学的な基礎的問題として，水溶性固定剤によっても青木の分類せる7物質を証明し得たとともに，その成績から可溶性固定液を使用しても被検物質が巨大分子の一部を形成せる場合は基材の固定が同物質の固定に他ならないとの Lison の考えを支持したい。なおフォルマリン固定液を使用せる場合は間質多糖類は結合織纖維上に附着凝集して認められ，純アルコール固定時の瀰漫性な微細顆粒状像とその所見を異にする。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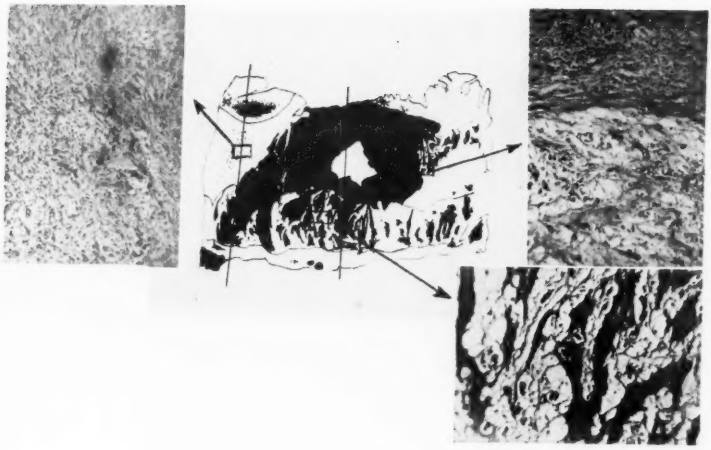


Fig. 9.

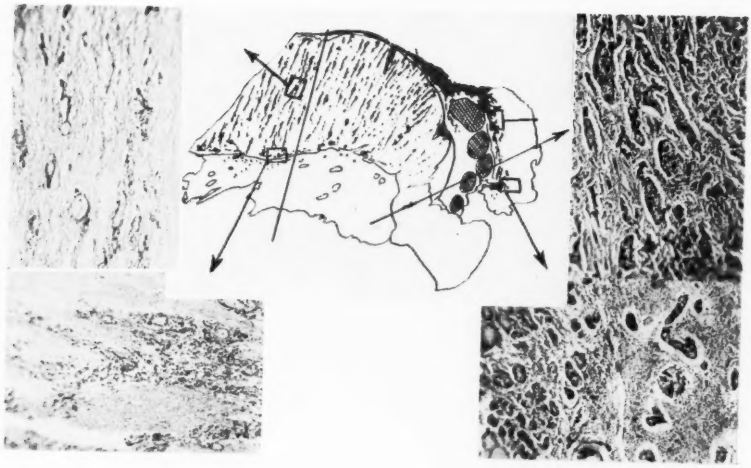


Fig. 10

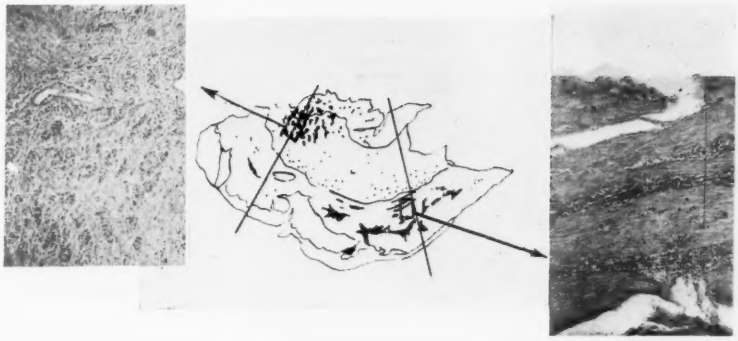


Fig. 11

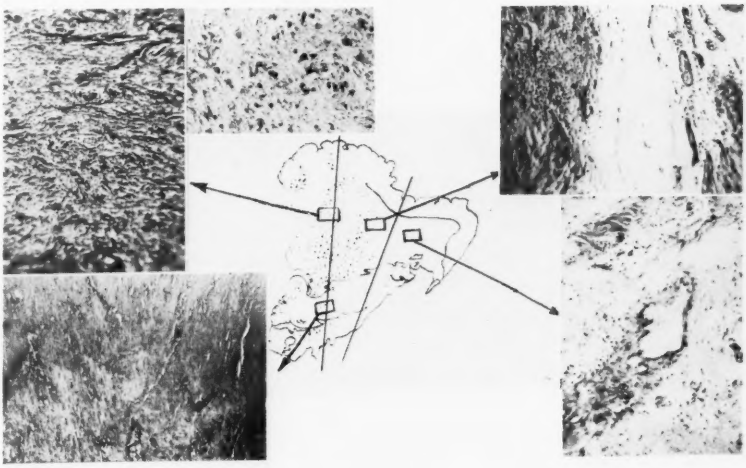
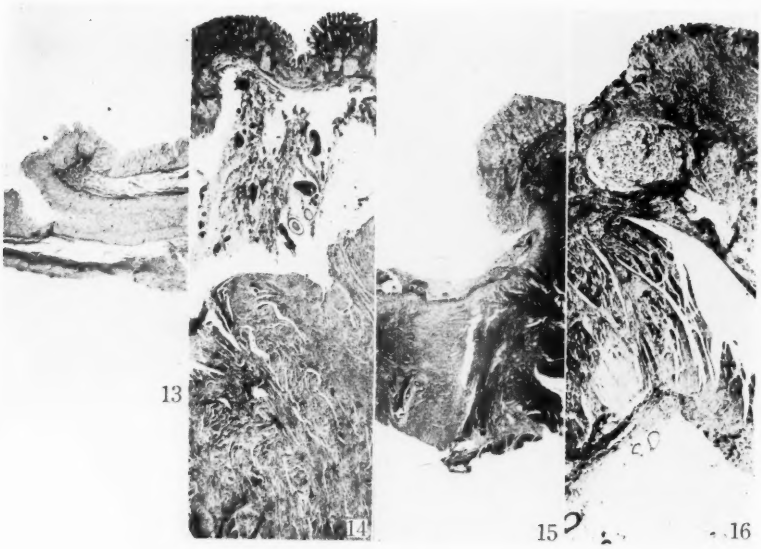


Fig. 12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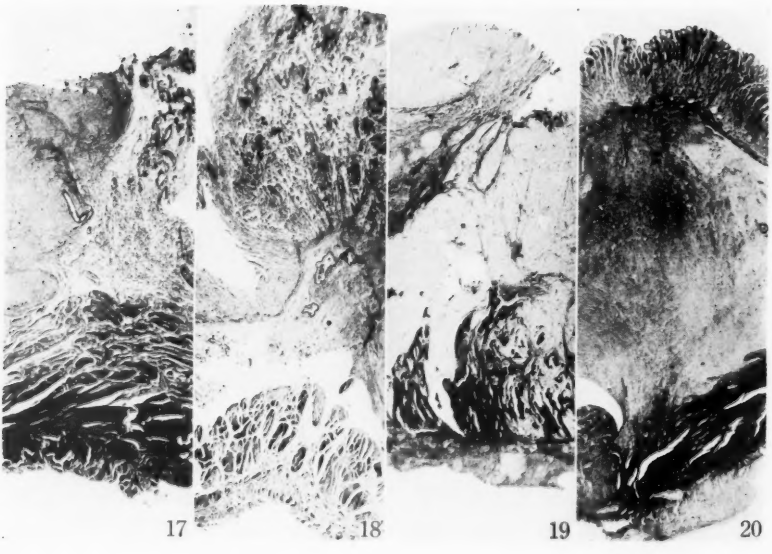
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[GANN, Vol. 46; December, 1955]

ON THE IMMUNOPATHOLOGICAL SPECIFICITY OF TUMOR CELLS. (Observed by Re- and Cross-transplantation of Rat Ascites Tumors in Mice)

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The problem of whether or not tumor cells really have specific antigenicity, besides species specific one common to animal strain from which the tumor originated, has been studied by many investigators. Thus, at present, it has been proved that antigenicity of tumor cells manifests itself only in animals heterologously transplanted with tumors, and not in homologously transplanted animals. However, much remains yet to be studied as to whether the immunity against tumor cells obtainable in heterologously transplanted animals is merely due to the genetical difference among strains of animals used or whether tumor cells themselves have certain specific antigenicity playing an additional role therein.

As previously reported, the present author and associates have several times carried out studies on the immunopathological phenomena in tumor by homo- or heterotransplantation of ascites tumor cells of rat (Yoshida sarcoma, Takeda sarcoma, MTK sarcoma, Hirosaki sarcoma and ascites hepatomata) and have found the existance in tumor cells of two kinds of antigenicity, one, species specific, which is common to animal strain in which tumor originated and the other, tumor type specific. The latter is different from the antigenicity of normal animal cells, being common not to all kinds of rat tumor, but common to those rat tumors which are provided with the same morphological and functional properties.

In the present study several rat tumors of ascites type have been used as materials, in view of the fact that in this type of tumor cells the sequence of immune responces can be easily followed.

Ascites Tumors of Rat used for the Study

1) Yoshida sarcoma type: Yoshida sarcoma; originated from the hybrid rat; monocytic character; chromosomes, 40±. MTK sarcoma I, II and III; originated from the Wistar strain (Makino). Hirosaki sarcoma; originated from the hybrid rat. These 5 ascites tumors have the same property in morphology and function.

2) Takeda sarcoma type: Takeda sarcoma; originated from the Yamashita strain of albino rat; histiocytic character; chromosomes, 80 ±. Usubuchi sarcoma; originated in the hybrid rat, morphologically and functionally almost the same as Takeda sarcoma, being, however, weaker in growth than the latter.

3) Ascites hepatomata: AAT I–II ascites hepatomata; derived from hybrid rats to which o-amidoazotoluene was given. DAB I–II ascites hepatomata; derived from hybrid rats in which dimethylaminoazobenzene was orally administered. These 4 ascites hepatomata have almost the same characteristics.

Animals used for Transplantation

1) Yamashita strain of albino rat
2) Gifu hybrid albino rats
} susceptible animals.

3) Wistar-Takeda strain of albino rat
4) Hybrid albino mice
5) Rabbits and goats
} insusceptible animals.

The susceptible rat strains are equally susceptible to all the eleven ascites tumors described above, dying of tumor in about ten days after abdominal inoculation of the tumor cells (14 days for hepatomata).

When inoculated in rats of the Wistar-Takeda strain, all these tumors first proliferate well, but about ten days after inoculation (about 14 days for hepatomata) the tumor cells suddenly disappear from the abdominal cavity by reason of the development of immunity which inhibit their proliferation, as reported before. The Wistar-Takeda strain is thus insusceptible to all these eleven tumors.

In insusceptible hybrid mice, these eleven tumors of rat proliferate well untill the fifth day after inoculation (8 th day in the case of hepatoma) in the abdominal cavity. Afterwards they spontaneously degenerate due to the immunity developing in the animals.

In the present experiment hybrid mice were used for heterologous transplantation or immunization, because, in these mice, the genetical differences such as exist between different rat strains and different rat tumors can be practically neglected.

For realization of the existance of immunity, findings were followed up comparatively in tumor-immunized mice and in mice in which inoculated tumor healed spontaneously, taking into account whether the tumor cells inoculated normally proliferate for 5 days after inoculation or whether they disappear within 1–2 days without showing any proliferation. This measure was applied, because whether tumor cells normally proliferate or inhibited of their proliferation *in vivo* supplies a more sensitive criterion for the existance of immunity than *in vitro* serological reactions.

Re- and Cross-transplantation

1) As shown in Table 1, mice which spontaneously recovered from Yoshida sarcoma could not be successfully reinoculated with the same sarcoma for about 300 days after natural healing. The retransplanted tumor cells disappeared almost completely within 24 hours without proliferation. This negative take is noted with

Table 1. Re- and Cross-Transplantation in Mice Cured from Yoshida Sarcoma.

	Tumors Transplanted	Number of Days after Imminization 50 100 150 200 300
Retransplantation	Yoshida sarcoma	
Cross-transplantation	MTK sarcoma	
	Hirosaki sarcoma	
	Takeda sarcoma	
	Ascites hepatoma	

○.....Negative Take ◑.....Incomplete Take ●.....Positive Take

Table 2. Re- and Cross-Transplantation in Mice Cured from MTK Sarcoma.

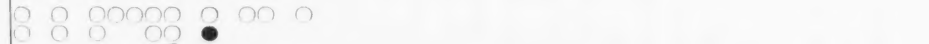
	Tumors Transplanted	Number of Days after Immunization 50 100 150 200 300
Retransplantation	MTK sarcoma	
Cross-transplantation	Yoshida sarcoma	
	Hirosaki sarcoma	
	Takeda sarcoma	
	Ascites hepatoma	

Table 3. Re- and Cross-Transplantation in Mice Cured from Takeda Sarcoma.

	Tumors Transplanted	Number of Days after Immunization 50 100 150 200
Retransplantation	Takeda sarcoma	
Cross-Transplantation	Yoshida sarcoma	
	MTK sarcoma	
	Hirosaki sarcoma	
	Ascites hepatoma	

an open circle in the table.

When mice which recovered from Yoshida sarcoma were cross-nioculated either with MTK sarcoma or Hirosaki sarcoma, a negative take was always observed during subsequent 200 days, just as in case of the reinoculation with Yoshida sarcoma.

The cross-transplantation of Takeda sarcoma into similarly treated mice was neither successful, the take being always negative for about 50 days after healing of Yoshida sarcoma. However, from the 50 th day after natural healing the cross-transplantation of Takeda sarcoma was not hindered, the take being almost as successful as in normal mice. Mice which recovered from Yoshida sarcoma betrayed the same attitude toward the cross-transplantation of ascites hepatomata as toward that of Takeda sarcoma. This positive take is noted with a black mark.

2) Mice which healed from MTK sarcoma resisted not only against the retransplantation of MTK sarcoma but also against the cross-transplantation of both Yoshida sarcoma and Hirosaki sarcoma for more than 200 days. But, in these mice, the cross-transplantation of Takeda sarcoma or of ascites hepatoma was hindered only for about 50 days. After this period of time the take of the latter two tumors was positive as in normal mice (Table 2).

From these observations it can be said that the immunity against transplantation of Yoshida sarcoma is perfectly consistent with that against MTK sarcoma and Hirosaki sarcoma. The immunity against proliferation of Takeda sarcoma and of ascites hepatomata as noticed in these mice is consistent with that against Yoshida sarcoma, MTK sarcoma and Hirosaki sarcoma only for 50 days, but this is not the case from the 50 th day on after natural healing from tumors.

3) Mice which spontaneously healed from Takeda sarcoma could not be successfully retransplanted with Takeda sarcoma for more than 200 days, as shown in Table 3, but the cross-transplantation of Yoshida sarcoma, of MTK sarcoma, of Hirosaki sarcoma or of ascites hepatomata into those mice was hindered only for 50 days. After this period the take of those tumors was almost always positive as in normal mice.

4) In accordance with those observations, mice which recovered from any one of the ascites hepatomata resisted the retransplantation of all the ascites hepatomata for a long period of time. But the cross-transplantation of Yoshida sarcoma and of Takeda sarcoma proved successful from the 50 th day on after healing, as in normal mice.

These results show that the 4 strains of rat hepatomata have a common antigenicity against retransplantation of different tumors, and that the antigenicity of Yoshida sarcoma and of Takeda sarcoma is only partially common to that of hepatoma.

5) As is demonstrated in Table 4, the control mice immunized with various

normal tissues (kidney, liver, erythrocytes, etc.) from tumor susceptible rats resisted the proliferation of all the rat ascites tumors. But the take was negative only for about 30 days after immunization. Even when an energetic immunization was carried out with normal liver, the take of hepatoma was no longer inhibited from the 30 th day on after immunization.

These facts demonstrate that the common resistance observed in the mice used against transplantation of all the ascites tumors during fifty days after natural healing from tumor is due to the common antigenicity of both the rat tumors and the normal tissues of tumor susceptible rat. This immunity, though persisting only for a short period, is considered to be species specific immunity.

The long-term resistance to tumor, not common to all the kinds of rat tumor, and specific not to each tumor, but to those types of tumor which have the same property, is probably due to the antigenicity specific to each type of tumors. This is considered as tumor type specific immunity.

From the standpoint of type specificity of tumor antigenicity, the eleven rat ascites tumors mentioned above can be classified into the following three different types.

I. Yoshida sarcoma type (Yoshida sarcoma, MTK I, II, III sarcoma, Hirosaki sarcoma)
II. Takeda sarcoma type (Takeda sarcoma, Usubuchi sarcoma)
III. Ascites hepatoma type (AAT hepatoma I, II, DAB hepatoma I, II)

Properties of the Tumor Antigens

1) When mice were repeatedly immunized with Yoshida sarcoma, Takeda sarcoma or normal tissues of susceptible rat, which were all previously lyophilized, they acquired only a common species specific immunity for about 20 days (Table 5). Even when mice were immunized with lyophilized Yoshida sarcoma, their resistance against the proliferation of Yoshida sarcoma was not notably accentuated, as compared with their resistance against Takeda sarcoma. This fact shows that species specific antigenicity remains still in the tumor cells and normal tissues even after they are subjected to lyophilization, while tumor type specific antigenicity apparently decreases following this treatment.

2) When mice were immunized with tumor cells previously treated with 5 % formol solution, they resisted, for about 20 days, only the inoculation of tumor of the same type as used for immunization.

As is demonstrated in Table 6, mice immunized with formoled Yoshida sarcoma hindered the proliferation of Yoshida sarcoma and of MTK sarcoma for about 20 days, but the take of Takeda sarcoma and ascited hepatoma was successful in these mice just as in normal mice.

Mice immunized with formoled MTK sarcoma hindered the proliteration of

Table 4. Transplantation in Mice Immunized with Fresh Tissue of Susceptible Rats.

Immunized with	Tumors Transplanted	Number of Days after Immunization: 5	10	15	20	25	30
Liver	Yoshida sarcoma	○ ○	○ ● ○	○ ○	○ ○	○	○○ ○ ● ●
	Takeda sarcoma	●○	◑	○ ○	○ ○		○ ●
	Hepatoma	◑○	○○	○ ○ ●	○○ ○	○○	◑ ● ●
Kidney	Yoshida sarcoma	○	○	●○○	○ ○○		○● ●●
	Takeda sarcome	○	○○	○○	●○○		● ●
	Hepatoma	○ ○	○	○ ○	○		●
Brain	Yoshida sarcoma		○		○○		○ ●
	Takeda sarcoma	●	○		○	○	◑ ●
	Hepatoma	○	○	○	○		●
Red blood corpuscles	Yoshida sarcoma			○○○○	○○○		● ●
	Takeda sarcoma	◑○○○○		○ ○ ●	○	○	● ● ●
	Hepatoma	◑○○	○○	◑○○	○○	● ◑	● ●
Serum	Yoshida sarcoma			○	○ ○ ○		●
Spleen	Yoshida sarcoma		○○○	○○	○		●○ ●
Testis	Yoshida sarcoma		○○	○○	○		○ ○○ ●

Table 5. Transplantation in Mice Immunized with Lyophilized Ascites Tumors.

Immunized with	Tumors Transplanted	Number of Days after Immunization 5 10 15 20 25 30
Lyophilized Yoshida sarcoma	Yoshida sarcoma	
	Takeda sarcoma	
Lyophilized Takeda sarcoma	Takeda sarcoma	
	Yoshida sarcoma	
Lyophilized Hepatoma	Hepatoma DAB I	
	Hepatoma AAT I	
	Yoshida sarcoma	
Lyophilized normal red corpuscles	Yoshida sarcoma	
	Takeda sarcoma	

Table 6. Transplantation in Mice Immunized with Formoled Yoshida Sarcoma.

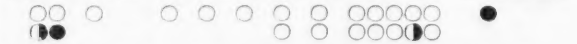
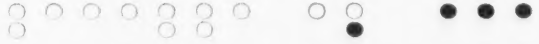
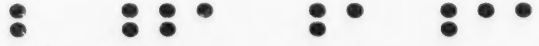
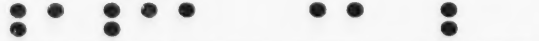
Immunized with	Tumors Transplanted	Number of Days after Immunization 5 10 15 20 25 30
Formoled Yoshida sarcoma	Yoshida sarcoma	
	MTK sarcoma	
	Takeda sarcoma	
	Ascites hepatoma	

Table 7. Transplantation in Mice Immunized with Formoled MTK Sarcoma.

Immunized with	Tumors Transplanted	Number of Days after Immunization 5 10 15 20 25
Formoled MTK sarcoma	MTK sarcoma	
	Yoshida sarcoma	
	Takeda sarcoma	
	Ascites hepatoma	

MTK sarcoma and of Yoshida sarcoma for about 20 days (Table 7) but not that of Takeda sarcoma and of ascites hepatoma. This finding agrees with that observed in mice immunized with formoled Yoshida sarcoma.

As shown in Table 8, mice immunized with formoled Takeda sarcoma inhibited the proliferation of Takeda sarcoma and of Usubuchi sarcoma for 20 days, but not that of Yoshida sarcoma type or of ascites hepatomata.

As shown in Table 9, mice treated with formoled ascites hepatoma, whichever of the 4 strains of hepatomata was used for immunization, suppressed the proliferation of all the strains of hepatoma for 20 days, but not that of Yoshida sarcoma and of Takeda sarcoma.

As control, as is presented Table 10, in mice which were immunized with formoled normal tissues and erythrocytes of susceptible rat all ascites tumors of rat took almost as successfully as in normal mice.

If mice were immunized with formoled normal liver of susceptible rat, the take of hepatoma was not notably hindered.

From these results it can be said that the species specific antigenicity common to all the ascites tumors of rat and the normal tissues of rat may be stable to lyophilization, while it is diminished by treatment with formol. On the contrary, the type specific antigenicity of tumor which decreases following lyophilization, has a characteristics to resist the treatment with formol. These facts indicate that these two kinds of antigen have their own physico-chemical properties different from each other. Analytical study on the tumor type specific antigen is now under way.

Experiments on the Strengthening of Antigenicity

As described above, mice treated with formoled tumor cells showed specific resistance against the inoculation both of tumors used for immunization and of tumors having the same properties, but the resistance lasted only for about 20 days. In view of this fact, studies are now being carryed out on the strengthening of the antigenicity of tumor antigens.

1) As presented in Table 11, mice immunized with formol treated tumor cells mixed with almina cream inhibited the proliferation of retransplanted tumor cells for more than 60 days, the takes of the other types of tumor were not inhibited in these mice, though not always positive.

2) Mice immunized with 5% trichloro-acetic acid treated (instead of formol) tumor cells (washing with saline is necessary after treatment) resisted the proliferation of the same tumor cells as used for immunization for about 30 days. On the contrary, the proliferation of the other types of tumor was not inhibited at all in those mice. The difference between the two types of tumor antigenicities in producing resistance to mice was more clearly noticed when the antigens were

Table 8. Transplantation in Mice Immunized with Formoled Takeda Sarcoma.

Immunized with	Tumors Transplanted	Number of Days after Immunization 5 10 15 20 25 30
Formoled Takeda sarcoma	Takeda sarcoma	
	Yoshida sarcoma	
	Ascites hepatoma	

Table 9. Transplantation in Mice Immunized with Formoled Ascites Hepatomata (AAT I-II and DAB I-II)

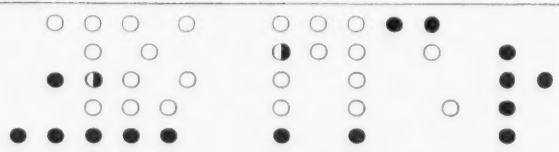
Immunized with	Tumors Transplanted	Number of Days after Immunization 5 10 15 20 25
Formoled hepatoma AAT I	Hepatoma AAT I	
	Hepatoma AAT II	
	Hepatoma DAB I	
	Hepatoma DAB II	
	Yoshida sarcoma	
	Takeda sarcoma	
Formoled hepatoma DAB II	Hepatoma DAB I	
	Hepatoma DAB II	
	Hepatoma AAT I	
	Hepatoma AAT II	
	Yoshida sarcoma	
	Takeda sarcoma	

Table 10. Transplantation in Mice Immunized with Formoled Organs of Susceptible Rats.

Immunized with	Transplanted Tumors	Number of Days after Immunization (5 / 10 / 15 / 20 / 25)
Formoled Liver	Yoshida sarcoma	●●●●● ●●●●● ●●●●● ●●● ● ●
	Takeda sarcoma	● ●● ●● ● ○ ●● ●
	Hepatoma DAB I	●○ ●● ● ● ● ● ●
Formoled Kidney	Yoshida sarcoma	● ● ● ● ● ● ● ●
	Takeda sarcoma	● ●◑ ● ● ● ●
	Hepatoma DAB I	● ● ● ● ● ●
Formoled Red blood Corpuscles	Yoshida sarcoma	● ● ● ● ● ● ● ● ● ●
	Takeda sarcoma	● ● ● ● ● ● ● ● ●
	Hepatoma DAB I	● ● ● ● ●

Table 11. Transplantation in Mice Immunized with Formoled Yoshida Sarcoma mixed with Almina Cream.

Immunization with	Tumors Transplanted	Number of Days after Immunization (10 / 20 / 30 / 40 / 50 / 60 / 70 / 80)
Formoled Yoshida sarcoma mixed with Almina Cream	Yoshida sarcoma	○○○○ ○◑○◑ ○○ ○ ○ ○ ○ ● ●
	MTK sarcoma	● ○ ○○○ ○ ● ○
	Hirosaki sarcoma II	○ ○ ○ ○
	Takeda sarcoma	● ● ● ● ◑ ◑ ● ● ●
	Usubuchi sarcoma	● ◑ ● ● ○ ●
	Hepatoma DAB I	● ● ● ● ● ● ● ● ●

treated with trichloro-acetic acid than with formol (Table 12).

Properties of the Antibodies against Tumor Cells

In order to obtain precise information on the properties of the antibodies against tumor cells, the following serological studies were carried out.

1) Susceptible rats immunized with tumor cells by repeated transplantations and excisions in the intracutaneous tissue inhibited the take of the same types of tumor as used for immunization, but not the take of the other types of rat tumor. The sera obtained from the immunized rats did not agglutinate the homologous tumor cells. However, when these tumor cells were mixed *in vitro* with the corresponding antisera and inoculated into the abdominal cavity of susceptible rats, they did not take at all, whether they were of the same tumor cells as used for immunization or of tumor cells of the same type. On the contrary, the other types of tumors proliferated well in the abdominal cavity, even if they were treated in the same manner (neutralization test). The electrophoretical patterns of the immune sera as traced by the Tiselius apparatus showed an increase in the gamma globulin fraction, while the decrease of the same fraction was noticed, when the immunized animals were desensitized by intraperitoneal reinoculation of corresponding tumor cells.

2) Insusceptible animals (rats and rabbits) which healed spontaneously from tumor of rat or those immunized with fresh tumor cells showed a short-term resistance of the same order against all the types of rat tumor, just as in the case of mice. From the 30 th day on after natural healing, however, the take of the other types of rat tumor not used for immunization were not suppressed, while the take of the tumor used for immunization was hindered for a long period of time. The sera obtained from these animals showed an apparent agglutination reaction not only towards corresponding tumor cells as used for immunization, but also towards those of other types. Erythrocytes and monocytes of susceptible rats were also agglutinated almost to the same titer. The electrophoretical pattern of the sera demonstrated a marked increase in gamma globulin. On the contrary, the decrease of the same fraction was noticed, when the immunized animals were desensitized.

3) The immune sera obtained from those insusceptible animals (rabbit and goat) excerted a strong cytotoxic action not only upon all the kinds of rat tumor, but also upon the normal tissues of susceptible rat, in accordance with the conception of reversed allergy.

4) After these sera were absorbed with protein of normal rat tissues, the presence of agglutinin could no longer be proved either towards all the tumor types or towards the normal free cells. But when these absorbed sera were injected, in rats, in the abdominal cavity previously inoculated with tumor cells,

or previously mixed with the same tumor cells *in vitro* and inoculated in the abdominal cavity, the tumor cells disappeared completely from the abdominal cavity, without injuring the animals, provided that the tumor cells used belong to the same tumor used for immunization or to tumor of the same type. However, this was not the case for the other types of tumor not used for immunization, either *in vitro* or *in vivo*.

These facts show the existance of two kinds of antibody in the sera of animals immunized with tumor cells; one is species specific, showing agglutination and cytotoxic action not only upon all the types of rat tumor, but also upon normal free cells and tissues of rat. The other is tumor type specific, showing no agglutination either with pathological or normal free cells of rat. This type of antibody has an injurious action only upon tumor cells of the same type as used for immunization, and not upon other types of rat tumor.

Consideration

As previously reported several times and as also described in the present paper, the existance in tumor cells of two kinds of antigenicity has been experimentally demonstrated. One is species specific and the other is tumor type specific.

In parallel to this fact, on the part of tumor susceptible and insusceptible animals, two kinds of resistance against proliferation of tumor cells have been separately realized *in vivo* by re- or cross-transplantation of tumor cells at a certain stage after the animals undergo natural healing from tumor inoculated. These two kinds of resistance are demonstrable dissociated from each other by selecting suitable animal strains for immunization experiment or by searching for a certain stage suitable for test after immunization with tumor cells.

In addition, the existance of two kinds of antibody in immune sera against tumor is demonstrable *in vitro* by means of the agglutination and neutralization tests with corresponding tumor cells or *in vivo* by treating tumor bearing animals with corresponding immune sera. These two kinds of antibody are also separately demonstrable by the absorption test with normal tissue protein from susceptible rats, while the two different antigenic factors in tumor cells are also shown by lyophilization, by formol treatment or by treatment with trichloro-acetic acid.

Now it is of course possible that certain genetical differences should exist among different rat strains or different tissue cells from which these eleven tumors originated. However, merely these genetical differences could not account for the production of such strong and characteristic type specific immunity to tumor as was demonstrated in the present study, in the mouse which is genetically quite different from the rat in which the tumor cells used have originated. This assumption would be reasonably endorsed by the facts that the susceptible rat strains are equally susceptible to all these eleven ascites tumors of rat, and

Table 12. Transplantation in Mice Immunized with Tumor Cells Treated with Trichloroacetic Acie.

Immunization with	Tumors Transplanted	Number of Days after Immunization 10 15 20 25 30 35
Yoshida sarcoma treated with Trichloroacetic Acid	Yoshida sarcoma	○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ◑ ● ●
		○ ○ ○ ○ ○ ○ ○ ○
	Hepatoma DAB I	○ ● ● ● ● ● ● ● ● ● ● ● ●
		● ● ● ●
Hepatoma DAB I treated with Trichloroacetic Acid	Hepatoma DAB I	● ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○
		● ○ ○ ○ ○ ○ ○ ○ ○
	Yoshida sarcoma	● ● ● ● ● ● ● ● ● ● ● ● ●
		○ ● ● ● ●

Table 13. Type Specific Antigenicity of Tumor.

Transplantation of / Immunization with	Yoshida Sarcoma Type			Takeda Sarcoma Type		Hepatoma Type			
	Yoshida sarcoma	MTK Sarcoma	Hirosaki sarcoma	Takeda sarcoma	Usubuchi sarcoma	AATI	AATII	DBAI	DBAII
Yoshida sarcoma	○	○	○	●	●	●	●	●	●
MTK sarcoma	○	○	○	●	●	●	●	●	●
Hirosaki sarcoma	○	○	○	●	●	●	●	●	●
Takeda sarcoma	●	●	●	○	○	●	●	●	●
Usubuchi sarcoma	●	●	●	○	○	●	●	●	●
Hepatoma AAT I	●	●	●	●	●	○	○	○	○
Hepatoma AAT II	●	●	●	●	●	○	○	○	○
Hepatoma DAB I	●	●	●	●	●	○	○	○	○
Hepatoma DAB II	●	●	●	●	●	○	○	○	○

that each immune serum prepared from any one of these tumors agglutinates all the eleven tumors as well as normal free cells of the susceptible rats always to the same titer. Thus, the difference between species specific antigenicity and type specific one of tumor cells as demonstrated in the present study would be scarcely attributable to the possible antigenic differences among animal strains or different organ tissues. Moreover, what is particularly worth stressing is first that the type specificity of tumors manifested itself selectively common to those tumors that have the same morphological and functional properties, whether they spontaneously originated in different hybrid rats or whether they were artificially produced, and, second, that the two kinds of antigenicity of the tumor cells used could be dissociated from each other by suitable chemical or physical treatment of the cells, indicating the possible presence in these tumor cells of two substances physico-chemically and antigenically different from each other.

From the standpoint of type specificity of tumor cells, the eleven ascites tumor strains used are classified into three different types, namely, Yoshida sarcoma type, Takeda sarcoma type and ascites hepatoma type. The morphology and function of these three tumor types are so characteristic of one another that the morphology of the cells of any given tumor can be easily and correctly assumed without help of the microscope, if the type of this tumor is immuno-pathologically determined. This is valid at least tor the eleven tumor strains studied.

As to whether or not specific antigenicity exists in tumors, negative opinions seems to be prevailing at present, except in case of virogenic tumors. However, the results of the present study clearly demonstrate the existance in tumors of type specific specificity, at least in case of the eleven tumor strains studied. This type specificity of tumor would play some role in the specific proliferation of cells of respective tumor types.

Experiments are now under way, in order to determine whether this type specificity of tumor is also demonstrable in human tumors and whether it can be used as a means for diagnosis and treatment of different kinds of tumors.

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要　旨

腫瘍細胞の免疫病理学的特異性

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癌細胞に特殊な抗原性が存するか否かを 11 系のラッテ腹水癌をマウスに異種移植して検した。用いた腹水癌は形態機能的に I) 吉田肉腫型 (吉田肉腫, MTK 1-3, 弘前肉腫), II) 武田肉腫型 (武田肉腫, 臼淵肉腫), III) 肝癌型 (AAT 1, 2, DAB 1, 2) の 3 型である。

これらの腹水癌をマウス腹腔に移植すれば5日前後(肝癌では8日)よく増殖し後免疫の発生によって急激に自然治癒し，その後は同癌の再移植，同型癌の交叉移植を 300 日以上強く阻止して take されない。しかし異型の腹水癌を交叉移植すれば治癒後 50 日までは明かに移植は阻止されるがそれ以降の移植は正常マウス同様阻止されない。対照として感受系ラッテ組織でマウスを免疫すれば 30 日間はすべてのラッテ腫瘍の移植を共通に阻止するが以降の移植はいずれも正常マウスに等しい。

すなわちラッテ癌自然治癒後の抗移植性免疫は 1) 短時日すべてのラッテ癌移植を共通に阻止する正常ラッテ組織免疫とも共通な種属特異性免疫と 2) 長期間上記3腫瘍型間に独立した抗移植性を示すラッテ正常組織と無関係な抗原に基く腫瘍型特異性免疫の2つの因子からなる。

ラッテ各癌を凍結乾燥，凍結融解してマウスを免疫すればすべてのラッテ癌移植を共通に 20 日間阻止し，正常ラッテ組織を同様に処理して免疫しても同一の結果を得る。すなわちかかる方法で腫瘍型特異抗原性は低下し，種属特異抗原性は安定である。

しかるにラッテ癌を5％のフオルモールあるいは5％の三塩化醋酸処理後水洗してマウスを免疫すれば免疫原癌と同型の癌移植を 20-30 日間強く阻止するが，異型癌の移植ははじめから正常マウス同様に成立する。対照として感受系ラッテ組織を同様処理して免疫しても各癌の take は阻止されない。すなわちかかる処理では逆に種属特異性因子は消失して腫瘍型特異性因子のみが残り，両抗原因子はその性状を異にすることを知る。

以上の事実からラッテ腹水癌細胞にはラッテ正常組織と共通な抗原性がある他になお各癌に特異な抗原性があり，この後者は同一の形態機能を有する一定の腫瘍型間にのみ共通で，正常組織および他型癌から独立した特殊な抗原性であると思われる。

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PURIFICATION OF THE LIVER CATALASE-REDUCING SUBSTANCE OCCURRING IN CANCER TISSUES, WITH SPECIAL REFERENCE TO ITS ACTIVITY

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INTRODUCTION

It was shown by Blumenthal and Brahn (1910) that liver catalase activity of tumor-bearing patients was remarkably low as compared with that of patients of other diseases. Similar results were derived by Rosenthal (1912) through his study of tumor-bearing animals. He stated that the emulsion of tumor tissues inoculated into peritoneal cavities of normal mice caused the depression of liver catalase activity in a similar range as was the case in tumor-bearing mice. This led him to the conclusion that certain toxic substance might be produced in tumor tissues which served to decrease the liver catalase activity of mice. Greenstein (1947, 1952) and his co-workers showed also the fact that the depression of liver catalase activity always occurred in tumor-bearing animals. But none of them have met with success to chemically isolate a liver catalase-reducing substance from tumor tissues.

Nakahara and Fukuoka (1948, 1949) were the first to discover the presence of a toxic substance called "toxohormone" in tumor tissues. They indicated that the toxohormone depressed liver catalase activity following peritoneal injection into normal mice. The toxohormone purified by them proved active in a dosage of 5 mg when injected into the peritoneal cavities of mice. The toxohormone is regarded as a substance of thermostable, non-heat-coagulable, water-soluble and alcohol precipitable polypeptid-like nature.

Kosuge (1952, 1954) one of the present authors, found a substance like the toxohormone in the ascites of tumor patients, and he was successful in extracting a substance of similar nature from tumor tissues. The substance reduced the liver catalase activity of mice at a dose of 5 mg.

In a recent paper (1954) was reported a successful purification of catalase-reducing substance in cancer tissues. Further information with additional data will be dealt with in this paper.

MATERIAL AND METHODS

Tissues of human carcinoma and Brown-Pearce rabbit carcinoma provided the

material for the present experiment. The activity of various tumor fractions was tested by injecting them into the peritoneal cavities of normal Swiss albino mice weighing 14~18 g. The liver catalase activity was examined 24 hours after injection. For estimation of liver catalase activity the potassium permanganate-method after Kosuge (1954) was applied.

Enzyme solution: Fifteen one hundredths g of fresh liver tissue was removed from a mouse killed by decapitation, throughly mashed and extracted with 15 cc of M 75 phosphate buffer of pH 7.0. Then the extract was spun and 1 cc of the centrifugal supernatant was diluted with 9 cc of the same buffer solution.

Substrate solution: It consists of 0.01 M H_2O_2 solution containing M 75 phosphate buffer.

One cc of the enzyme solution was added to 49 cc of the substrate solution at 0°C. Each 5 cc of the reaction mixture was pipetted into 2 cc of 10 % H_2SO_4 at 4′, 5′ and 6′ after adding of the enzyme solution. The solution thus prepared was titrated with N/25 potassium permanganate to determine the remaining quantities of peroxide. If the used cc of potassium permanganate for the titration of mixed solusion be b, the used cc of potassium permanganate at zero time be a, and the reaction time be t, so the catalase activity becomes $K=\frac{1}{t}\log\frac{a}{b}\times 1000$. K is the averaged value of the three at 4′, 5′ and 6′.

Experiments

1) Liver catalase activities of normal mice range from 37.7 to 20.1 as given in Table 1.

Table 1. Liver catalase activity of normal mice.

Mouse No.	Catalase activity	Mouse No.	Catalase activity	Mouse No.	Catalase activity
1	37.7	12	29.3	23	25.5
2	37.7	13	29.1	24	25.5
3	35.3	14	28.4	25	24.6
4	35.3	15	28.4	26	24.5
5	34.0	16	27.5	27	24.2
6	32.7	17	27.5	28	24.2
7	32.0	18	27.4	29	22.8
8	31.6	19	27.4	30	20.2
9	31.1	20	27.3	31	20.1
10	29.6	21	26.7		
11	29.4	22	26.5		

2) Brown-Pearce's rabbit carcinoma

i) Alcohol precipitate

Three carcinoma-bearing rabbits were sacrificed 25 to 30 days after transplantation. The tumor tissues i. e., the testicles and metastatic lymphglands, which grossly showed little or no necrosis, were homogenized with masticator. The homogenate was heated for 30 minutes in a boiling water bath under the pressure decreased by means of the vacuum pump. Then 5 cc of hot water was added per 1 g of original weight of cancer tissues. The contents were heated again for about one hour in a boiling water bath under continuous stirring, under such control of the pH of the contents as to keep it at about 7.0. The extract was filtered with gauze and concentrated to about 1/10 volume by use of the hot water bath. The concentrated extract was regulated to pH 7.5 by adding N/10 NaOH, and then was chilled to 0°C. The extract was centrifuged and the supernatant liquid was adjusted to pH 7.0 with N/10 HCl. Absolute alcohol was then added to the amounts of twice volume of the filtrate. The precipitate obtained by centrifugation at 3000 r. p. m. for 10 minutes was washed with absolute alcohol and ether. Thus the fraction was obtained as yellowish white powder; it is called 'Alcohol precipitate'. The yield of the Alcohol precipitate amounted to about 6~10 mg per 1 g of fresh tumor tissues.

The Alcohol precipitate was dissolved in water adjusting the pH to be 7.5. The insoluble material was removed by centrifugation, then the supernatant yellowish solution was corrected to pH 7.0. This solution was injected into the peritoneal cavity of a normal mouse. The volume of the solution at injection was regulated at 1 cc. Approximately 24 hours after injection, the mouse was killed by decapitation, then the liver catalase activity was immediately determined. The results are as shown in Table 2.

Table 2. Effect of Alcohol precipitate, from the experiments with Brown-pearce's carcinoma.

Tissue No.	Amount injected, mg	Mouse No.	Catalase activity
1	10	1	19.4
		2	22.3
	15	3	12.2
2	10	4	13.4
		5	13.6
3	10	6	9.9
		7	13.9
	1	8	27.0
		9	25.9
		10	33.7

These data show clearly that the Alcohol precipitate caused the reduction of

liver catalase activity at a dose of 10 mg, while there was no change at a dose of 1 mg.

ii) Copper precipitate

Further purification of catalase-reducing substance from active Alcohol precipitate was attempted following Nakahara-Fukuoka's procedure (1950). Namely, the yellowish solution obtained from Alcohol precipitate was adjusted to pH 7.0, and to it was added 3% $CuSO_4$ solution in proportion of 1% in its final concentration. The green precipitate thus obtained was centrifuged and washed with N/10 HCl until the copper reaction had disappeared. Thus a grayish white precipitate was obtained. It is called 'Copper precipitate 1'. Twenty to thirty mg of this precipitate 1 were obtained from 100 mg of the Alcohol precipitate. This precipitate was proved to be active in so small a dosage as 2 mg. The results are shown in Table 3.

Table 3. Effect of Copper precipitate 1, from the experiments with Brown-Pearce's carcinoma.

Tissue No.	Amount injected, mg	Mouse No.	Catalase activity
1	2	1	14.3
		2	13.3
		3	9.7
		4	14.6
2	2	5	14.2
	1	6	22.3
		7	19.7

Next the Copper precipitate 1 was dissolved in water, adjusting the pH to be 7.5. The amount of insoluble material was smaller than that of the Alcohol precipitate. The supernatant was corrected to pH 7.0 and to it was added 3% $CuSO_4$ solution, as described above. The precipitate was more intensely decolored than the Copper precipitate 1. About 70 mg of such precipitate was obtained from 100 mg of the latter. It is called 'Copper precipitate 2'. This fraction was proved to be effective at a dose of 1 mg, but inactive at a 0.2 mg dosage.

Table 4. Effect of Copper precipitate 2, from the experiments with Brown-Pearce's carcinoma.

Tissue No.	Amount injected, mg	Mouse No.	Catalase activity
1	1	1	8.9
		2	11.4
	0.2	3	25.0
		4	27.5
		5	39.5

The data given in Table 4 show the effect of Copper precipitate 2.

iii) KNA

As described below, Copper precipitate 2 showed in qualitative tests not only a strong positive biuret reaction but also a positive orcin-HCl reaction, along with a maximum absorption at 260 mμ wave length according to Shimazu's QB 50 spectrophotometer. An attempt was then made to separate this powder into a nucleic acid and a protein fraction. Copper precipitate 2 was dissolved by means of the procedure already described. The solution was adjusted to pH 7.0 when a small amount of precipitate came under study. An equal volume of cold 10% trichloroacetic acid was then added to it at a temperature of 0°C. After centrifugation, an equal volume of 5% trichloroacetic acid was added to the precipitate, and the mixture was heated for 15 minutes at 90°C. After the contents were chilled to 0°C, the precipitate was centrifugated at 3000 r. p. m. for 15 minutes. The precipitate was washed twice with absolute alcohol and ether. The yield thus produced was a yellowish white powder. On the other hand, 5 volumes of cold absolute alcohol was added to the supernatant. The precipitate and the supernatant solution were separated by centrifugal force. The former was washed twice with absolute alcohol and ether (KNA-1). The latter was evaporated, dried by means of a freezing method, and washed with alcohol and ether (KNA-2). The substances obtained from both fractions were snow-white in color. Their aqueous solution was colorless and transparent in nature. Of these three fractions serarated from Copper precipitate 2, the fraction of yellowish white color may probably be a protein fraction and the other two a nucleic acid fraction. They were called KNA-1 and KNA-2, respectively. It was shown that 8 mg of the protein fraction, 4 mg of KNA-1 and 6 mg of KNA-2 were produced from

Table 5. Effect of KNA-1, from the experiments with Brown-Pearce's carcinoma.

Tissue No.	Amount injected, mg	Mouse No.	Catalase activity
1	0.2	1	16.9
		2	14.4
		3	13.9
2	0.2	4	16.1
		5	20.7
	0.1	6	18.5
		7	16.8
		8	12.7
		9	16.6
3	0.2	10	15.6
		11	17.3
		12	13.9

70 mg of Copper precipitate 2.

The influence of these fractions on liver catalase activity was observed for each and the results are shown in Tables 5, 6 and 7.

Table 6. Effect of KNA-2, from the experiments with Brown-Pearce's carcinoma.

Tissue No.	Amount injected, mg	Mouse No.	Catalase activity
3	0.4	1	17.9
		2	18.5
	0.2	3	14.2

Table 7. Effect of protein fraction, from the experiments with Brown-Pearce's carcinoma.

Tissue No.	Amount injecten, mg	Mouse No.	Catalase activity
1	1.5	1	22.6
		2	21.1
2	1.0	3	26.3
		4	24.7
		5	16.9
		6	20.8
3	1.0	7	26.3
		8	24.4
		9	28.1

It was found that the nucleic acid fractions KNA-1 and KNA-2, were active in so small dosage as 0.1 to 0.2 mg, while the protein fraction was inactive at a dose of 1.0 to 1.5 mg.

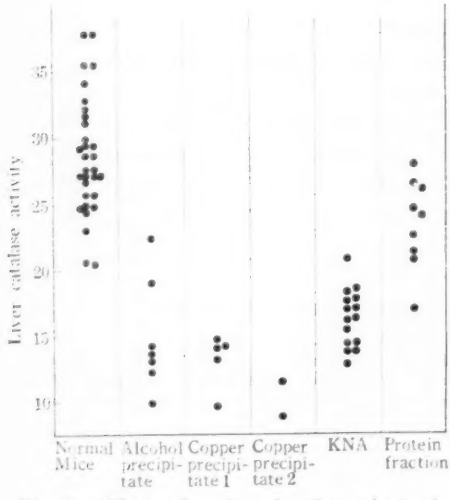

Fig. 1. Effects of various fractions obtained from Brown-Pearce's rabbit carcinoma.

The effects on liver catalase activity of various fractions obtained from the Brown-Pearce's rabbit carcinoma are illustrated in the form of a summary in Figure 1.

3) Human carcinoma

As material human cancer tissues of lung and stomach were used. Fractions of a similar nature were produced by the use of the same method as for the rabbit carcinoma above described. The fraction yielded in volume as much as the Brown-Pearce's rabbit carcinoma. The liver catalase activity of mice was examined by the

peritoneal injection of these fractions. The results are given in Table 8, and summarized in Figure 2.

Table 8. Effects of various fractions, from the experiments with human carcinoma.

Tissue	Fraction	Amount injected, mg	Mouse No.	Catalase activity
Cancer of lung	Alcohol precipitate	10	1	13.2
			2	20.6
	Copper precipitate 2.	1	3	19.3
			4	16.3
	KNA-1	0.2	5	21.9
			6	16.2
	Protein fraction	1	7	31.0
Cancer of stomach	Alcohol precipitate	10	8	16.7
			9	21.0
	Copper precipitate 2.	1	10	19.5
			11	20.0
	KNA-1	0.2	12	19.9
			13	16.4
			14	19.7
	Protein fraction	1	15	36.4
			16	24.9

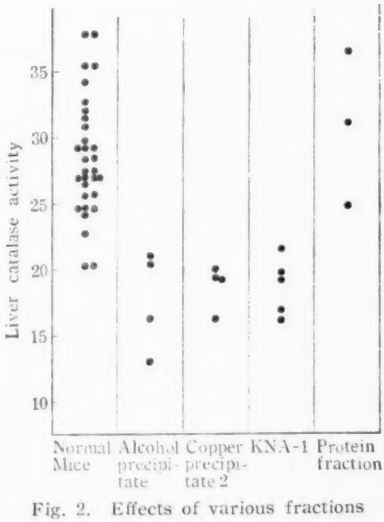

Fig. 2. Effects of various fractions obtained from human carcinoma

4. Fractionation of control tissues.

For the control experiment Alcohol precipitate was prepared from the normal rabbit liver and the liver of the persons who had died of non-cancerous diseases (meningitis tuberculosa, accidental death), and from the non-metastatic liver removed from one dead of gastric cancer.

Yields of the Alcohol precipitate were about 380 mg from 90 g of fresh rabbit liver. This fraction was proved inactive at a dose of 30 mg. Three hundred mg of this precipitate were dissolved in water, and 3% $CuSO_4$ solution was added. The precipitate thus obtained was washed with N/10 HCl. The volume of the precipitate was reduced as the copper reaction disappeared. The fraction thus obtained was yellowish brown in color, being 3 mg in amount. The mice which received the injection of this fraction did not show any appreciable

change in liver catalase activity at all. The results are as shown in Table 9. The fractions prepared from human control tissues were nearly the same in volume as those from the normal rabbit liver. As Table 9 shows, the liver catalase activity shows no decrease after their application. Unfortunately further trials were impossible, since volume of this fraction was too small.

Table 9. Effects of fractions obtained from control tissues.

Tissue	Fraction	Amount injected, mg	Mouse No.	Catalase activity
Rabbit liver	Alcohol precipitate	30	1	22.7
			2	22.6
	Copper precipitate 1	1	3	26.1
		2	4	35.2
Liver of accidental death man	Alcohol precipitate	30	5	24.3
			6	26.7
	Copper precipitate 2	3	7	26.2
Liver of meningitis tuberculosa	Alcohol precipitate	30	8	28.7
	Copper precipitate 2	3	9	28.8
			10	25.0
Liver of gastric cancer	Alcohol precipitate	30	11	40.9
	Copper precipitate 2	3	12	30.5
			13	29.5

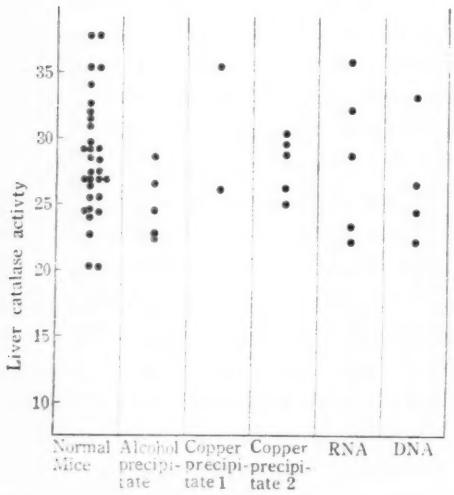

Fig. 3. Effects of various fractions obtained from control tissues and of RNA, DNA obtainable on the market.

5) Experiments with RNA and DNA obtainable on the market.

Further, some experiments with ribonucleic acid and desoxyribonucleic acid obtainable on the market were carried out, since the active substance in cancer tissues was found to be present in the nucleic acid fraction. The mice which received the injections of RNA and DNA at a large dosage such as 30 mg showed no decrease of liver catalase activity at all. The results are as shown in Table 10, and in Figure 3.

It must be mentioned that the mice receiving injection in the fore-

Table 10. Effect of RNA, DNA obtainable on the market.

Material	Amount injected, mg	Mouse No.	Catalase activity
RNA	30	1	28.6
		2	32.3
		3	22.4
	15	4	35.8
		5	23.1
DNA	30	6	26.6
		7	24.4
	15	8	33.1
		9	22.1

going experiments were all apparently as healthy as the untreated ones, and that there was no mouse which died within 24 hours atter injection.

6) Qualitative tests

The qualitative tests ot the substances obtained from cancer and control tissues were made with the use of their 0.1% aqueous solution. The Alcohol precipitate was positive in the biuret, Molisch and orcin-HCl reactions, whereas KNA gave a negative biuret reaction and a strong positive Molisch reaction. However, KNA revealed the presence of protein in very small quantity when examined by the method using chloroform-gel. On the other hand, the protein fraction was strongly positive in the biuret reaction, giving a negative orcin-HCl reaction. A Dische's diphenyl-amine reaction was found to be negative with all fractions.

7) Photological absorption

The absorption of the catalase reducing fraction by the ultraviolet rays was tested through the application of the spectrophotometer. Both the Alcohol precipitate and KNA exhibited a maximum absorption at 260 mμ wave length, in striking contrast to the protein fraction where no such absorption was observed. Thus there is no difference between the fractions from cancer and control tissues so far as the present study was concerned. This is interesting in contrast to the fact that the respective biological activities of the two were found to be highly different.

Discussion

It has been already learned that the reduction of liver catalase activity is a significant biochemical feature in tumor-bearing animals. There are many who confirmed the presence of a liver catalase activity depressing substance in variaus cancer tissue (Nakahara, Greenfield et al., etc.). Successful work has been done by Nakahara and Fukuoka (1950) in the purification of the active substance called toxohormone; this substance is active for a mouse in such a small dosage as

5 mg. The results of the present experiments serve to a great extent to confirm those of Nakahara and Fukuoka, and reveal that the substance purified through the procedures given in the foregoing pages is active in such a very small dosage as 0.1 to 0.2 mg for a mouse. It is remarkable that the application of similar procedures with normal tissues has failed to yield such an active substance. It is therefore highly probable that the fraction here dealt with would be specific to cancer tissues, and that it is the most highly purified one ever obtained.

Next, a comparison is made between the substance here obtained and the toxohormone reported by Nakahare and Fukuoka. Comparing the two substances of Alcohol precipitate obtained in this study with the pooled alcoholic precipitate obtained by Nakahara and Fukuoka, the former are about 1/10 of that of the latter in yield, but the former are 10 times as great as the latter in activity. Alcohol precipitate was found to be inactive at a dose of 1 mg, and the Copper precipitate 2 was active at a dose of 1 mg, though the latter is less in yield than the Alcohol precipitate. Correlation between the decrease in yield and the increase in activity was the same in the case of KNA. The authors received the impression that the active substance may increasingly become pure as fractionation advances. Repeated attempts in purification caused the production of a substance which is active at such a small dosage as 0.1 mg.

In the photological test, the active substance here obtained exhibited a maximum absorption by the ultraviolet rays at about 260 mμ wave length. This evidence is of special interest in reference to the conclusion of Greenfield and Meister (1951) that there was no correlation between the activity and the absorption at a similar wave length. The extinction of KNA at 260 mμ was similar to that of the Alcohol precipitate. However, Alcohol precipitate and Copper precipitate 2, both obtained from control tissues, were inactive, though they showed a maximum absorption at 260 mμ wave length, being the same in the strength of their extinction as that of the cancer material. In the course of fractionation, yields of the control fractions were extremely decreased, so that no substance corresponding to KNA could be obtained. This evidence induces the idea that Alcohol precipitate should contain the inactive nucleic acids which would be separated from the active substance in the course of purification. The qualitative tests conducted in this study seem to indicate that KNA may be a substance which is composed chiefly of ribonucleic acid. But it is impossible to conclude at present that the active substance is a nucleic acid only, due to the presence of protein in KNA after the chloroform-gel method, though it is minute in amount. So far as the authors are aware, there has been presented no evidence to show that the tumor tissues differ from the normal tissues in composition and kind of the nucleic acid. Considered from the fact that the growth of malignant tissues

differs from that of normal tissues, it may be probable that the malignant tissues should contain the nucleic acid specific to them. But this does not mean that KNA contains all of the liver catalase reducing factors to occur in cancer tissues. Probably some other active substance or substances, may exist in it. Based on the data derived from the present investigation, the authors' view is that KNA-fraction is active in liver catalase activity. Probably the fraction obtained in this study is one of the most active substances ever reported.

Summary

The catalase reducing substance was secured from the tissues of Brown-Pearce's rabbit carcinoma and of human carcinoma.

1) The crude Alcohol precipitate was active at a dose of 10~15 mg, but inactive at a dose of 1 mg.

2) The active substance was precipitable with $CuSO_4$. The fraction once precipitated with $CuSO_4$ (Copper precipitate 1) proved to be active at a dose of 2 mg, and the faction precipitated twice with $CuSO_4$ (Copper precipitate 2) was active at a dose of 1 mg, while experimental animals showed no reaction at a dose of 0.2 mg.

3) Of the two fractions such as nucleic acid fraction (KNA) and a protein fraction derived from Copper precipitate 2, the former reduced liver catalase activity at a dose of 0.1~0.2 mg, but the latter proved inactive at 1.5 mg.

4) One-tenth% aqueous solution of KNA showed an intensely positive orcin-HCl reaction, but was negative in biuret reaction. It exhibited a maximum absorption in ultraviolet rays at about 260 mμ wave length, while the solution of the protein fraction showed at the same concentration the inverse reaction without showing any special absorption at 260 mμ.

5) Of the fractions obtained from the control tissues, both Alcohol procipitate and Copper precipitate was inactive at doses of 30 mg and 3 mg, respectively. The RNA and DNA obtainable on the market proved also inactive at a dose of 30 mg.

6) There was no apparent difference in amount of the Alcohol precipitate between the cancer and control tissues. But the Copper precipitate fraction derived from control tissues was very scanty in amount compared with that from cancer tissues.

7) The fraction corresponding to the KNA has not been obtained as yet from non-cancerous tissues.

8) Based on the data derived from the present investigation, the conclusion may be reasonable that the KNA fraction is one of the liver catalase depressing substances in cancer tissues, and that it is the most pure substance of this character, being composed chiefly of ribonucleic acid.

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要　旨

癌組織中の肝カタラーゼ減少性物質の純化に関する研究

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癌組織より注射することにより，廿日鼠の肝カタラーゼを減少せしめる物質を分離した。

1. 組織を減圧加熱，加溜水加熱抽出後，アルコール，硫酸銅処理を行い，さらに三塩化醋酸にて主核酸部分と主蛋白部分に分離した。分劃物は分劃が進行するにつれ，より少量で肝カタラーゼ活性度を減弱せしめた。すなわち最終産物の主核酸部分 (KNA と名付けた) は，0.1 mg で有効 (他方，主蛋白部分は 1.5 mg でも無効) であった。

2. KNA は雪白色の粉末で，水溶性 (溶液は無色透明) にて，その 0.1% 水溶液は，ビューレット反応，ニンヒドリン反応およびヂフェニールアミン反応陰性にて，モーリッシュ反応，オルチン－塩酸反応強陽性である。紫外線吸収試験により 260 mμ 附近に最大吸収を示した。よって KNA は，リボ核酸を主体とし，微量の蛋白を含有する (クロロホルムゲル法にて僅微の蛋白の存在を認めた) 物質と考えられる。

3. 非癌の上皮細胞組織からの分劃では，硫酸銅処理にて収量が極めて減少し，KNA 相当分劃物の収得は未だ成功していない。

4. 市販の RNA, DNA 注射群では，肝カタラーゼ活性度に無影響である。

[GANN, Vol. 46; December, 1955]

PRODUCTION OF RAT SARCOMA BY INJECTIONS OF PROPYLENE GLYCOL SOLUTION OF M-TOLUYLENEDIAMINE
(Plates XXIII and XXIV)

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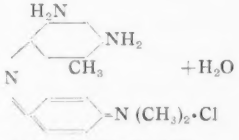
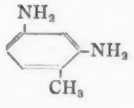
In 1954, reporting on experimental production of rat sarcoma by injections of toluyleneblue [I] I (1) expressed the opinion that m-toluylenediamine [II] (2) (3) (4) (5) (6) (7) may also act as a carcinogen, in spite of the fact that the substance is now generaly regarded as non-carcinogenic.



[I] Toluylene blue



[II] m-Toluylenediamine

An experiment has since been carried out using propylene glycol, instead of water, as solvent, with the surprising result that sarcoma developed in 100% of rats. Preliminary account of this experiment will be given in the following lines.

Carcinogenic activity of m-toluylenediamine has been tested in the past by many authors (Kinoshita (8) 1936, Uji (9) 1936, Watanabe (10) 1937, Okada (12) 1936, Nagaoka (13) 1935, Maruya (14) 1937, Shirakabe (15) 1937, Ogata (16) 1913, Ōno (17) 1931: 1933, Kaminuma (18) 1931, Rabl (19) 1935, Hayashi (20) 1939, Fieser (21) 1938), by feeding experiments which showed that this substance produces liver cirrhosis and sometimes liver adenomas in rat and mouse and icterus in dog, but no indisputable malignant tumor was produced. Yoshida, Shimauchi and Kinn (11) inserted m-toluylenediamine in collodium sack into the urinary bladder of the rat with view to producing bladder tumors, but without success.

Repeated subcutaneous injections of m-toluylenediamine into rats were tried by Nagata (22) (23), who used 1% waterly solution in doses of 0.5 to 1.0 cc for the period of 41 days, the number of injections varying from 3 to 40, and the amount of m-toluylenediamine from 15 to 300 mg. No tumor development was found in this experiment. Later, Isaka (24) reported a single case of myosarcoma which developed after repeated injections of 0.4 % waterly solutions of p-toluylenediamine which is an isomer of m-toluylenediamine demonstrated by Hashimoto to be a decomposition product of the carcinogenic azo-dye, o-aminoazotoluene (26).

In this experiment, the sarcoma developed after a period of 378 days, involving

72 injections, but only in a single rat which survived this period.

o-Aminoazotoluene　　o-Toluidin　　p-Toluylenediamine

Material and Methods

Experiment was started with 20 normal rats of a mixed strain from the Saitama Prefecture, all weighing around 200 g. They were kept in wire cages in groups of five each, and were maintained on the usual laboratory diet of whole wheat with occasional supply of dried fish and green vegetables.

The preparation of m-toluylenediamine (Alpha-diaminotoluol: $C_6H_3 : CH_3 : NH_2 : NH_2 = 1 : 2 : 4$) used in the experiment was the highest purity product of the Tokyo Kasei Industry Co., and propylene glycol ($CH_3CHOH \cdot CH_2OH$) was a product of the Kanto Chemical Co., also of the superior quality.

m-Toluylenediamine was dissolved in propylene glycol at the concentration ot 0.4 percent, and was injected subcutaneously on the back of the rats in 0.5 cc amounts. Injections were repeated once every week, and were delivered into as nearly the same site as possible. m-Toluylenediamine solution was prepared freshly every time, and sterilized by heating before injection.

Results

Of the 20 rats, with which the experiment was started, 9 rats survived the injection period of 246 days, receiving 28 injections during the period. Sarcoma developed in all of these 9 rats (Table 1).

The injections of m-toluylenediamine was not well tolerated, and as many as 11 of the 20 rats died within 8 months after the beginning of the injections. These rats showed no marked tissue changes which seemed significant. In the remaining 9 rats, subcutaneous connective tissue became thickened and small hard consolidations of various sizes became palpable during the 8th to 12th months. These consolidations gradually turned into irregular nodules, which, then, rapidly grew into large tumors. The tumors were at first more or less diffuse, but as they increased in size they became clearly demarkated from the surrounding connective tissue. The tumor, once developed, grew very rapidly to a very large size, and soon killed the animal.

No. 1. On the 246th day a hard nodule of the size of the small finger tip was found, which grew to the size of thum-tip by the 253rd day. The tumor grew very rapidly and killed the animal on the 297th day. The surface of the tumor was necrotic and ulcerated.

No. 2. The tumor was found as a hard nodule of the size of small finger-tip

Individual records of these 9 rats are as follows:

on the 321st day. The tumor reached the size of 4.1×3.5×1.9 cm on the 335th day. Animal died on the 348th day. This tumor was soft and showed haemorrhage and necrosis in the central part.

No. 3. A hard nodule of the size of small-pea was palpated on the 335th day, which attained the size of the small finger-tip by the 352nd day. On the 361st day the animal was found in very poor physical condition and was killed.

No. 4. On the 352nd day a hard nodule of the size of small-pea was palpated. The animal died on the 364th day with the tumor measuring 4.5×2.1×1.2 cm.

No. 5. A hard nodule of the size of small-pea, first palpated on the 352nd day, grew to the size of 4.4×3.6×2.5 cm on the 374th day. The animal was found very weak on the 384th day and was killed.

No. 6. A nodule measuring 3.2×2.2×1.7 cm was palpated on the 374th day. On the 396th day the animal was killed in very weakened condition.

No. 7. A small finger-tip sized nodule, first palpated on the 374th day, reached the size of 2.9×2.7×1.4 cm on the 388th day. The animal died on the 412nd day.

No. 8. On the 403rd day a small finger-tip sized nodule was found which grew to thum-tip size by the 412nd day. The animal was killed on the 431st day in very weakened condition.

No. 9. A small-pea sized nodule first palpated on the 412nd day, grew to a small finger-tip size by the 423rd day. The animal was killed on the 452nd day with the tumor measuring 4.3×3.2×2.5 cm.

Table 1

Rat No.	Sex	No. of days survived		Total amount injected		No. of injections	Body weight (Final)	Tumor size (cm)
		After first injection	After discontinuing injection	m.T. (mg)	P.G. (cc)			
1	male	297	44	60	15	29	350	9.3×6.3×4.5
2	male	348	27	72	18	35	340	7.8×5.8×4.6
3	male	361	9	76	19	37	270	4.4×3.7×2.2
4	fem.	364	12	76	19	37	180	4.5×2.1×1.2
5	male	384	32	76	19	37	352	5.8×5.4×3.7
6	fem.	396	44	76	19	37	220	7.3×4.7×2.9
7	fem.	412	38	78	19.5	38	270	6.4×4.1×3.5
8	fem.	431	28	84	21.5	41	171	4.7×3.2×2.5
9	fem.	452	23	90	23.5	44	235	4.3×3.2×2.5

Findings at Autopsy

All these tumors were found in the subcutaneous tissue at the site of the injections, the shape being semi-spherical to oblong with some irregularities. They were mostly elastic hard but some of them were softer. The cut surface was

generally pearly white, occasionally with pinkish or redish areas due to haemorrhage. The tumors were thinly incapsulated, clearly defined from the surrounding tissue to which they were slightly adherent. The basal part of the tumor was tightly adherent to the underlying tissue.

No macroscopic metastasis was found in any of the cases.

No significant change was noted in internal organs at autopsy. The liver was generally atrophic and histologically showed some hyperaemia and increase of Kupffer's stellate cells. Sometimes, in later stages, vacuolar degeneration of liver cells was noted. No change in the nature of liver surface, color, or consistency was recognized in gross and no cirrhotic change was recognized histologically. Spleen showed hyperaemia and sclerotic atrophy. Lymphnodes showed the proliferation of lymphocytes and histiocytic reticulum cells; blood vessels were dilated, and occasionally fibrosis and granulation formation were encountered. However, all these changes may be regarded as of no special significance in connection with the local sarcoma production.

Transplantation

Attempts to transplant the tumor to normal rats were made in the cases of No. 1, No. 2 and No. 5. In the cases of Nos. 1 and 2, tumor tissus for transplantation was taken from animals that have been dead, and probably for this reason transplantation did not succeed. The transplantation from No. 5 took in 70 % of the animals in the first generation and 20 % in the second. Further transplantation was not made.

Histology of the Tumors

Histological examination confirmed the tumor to be undoubted sarcoma in every case. Although each tumor seemed to show more or less characteristic histological pattern, individuality of the tumor was obscured by the intermixture of various histological features in different parts of any single tumor.

The cells composing tumor are arranged in disorder, often in bundless or fascicles of various sizes which run in every possible direction. The cells themselves are mostly spindle cells of plump or slender type. In a single case (No. 9) there were areas of medullary growth of polymorphic roundish cells surrounded by the usual spindle type cells. Nuclei are oblong or fusiform, rich in chromatin. Nucleolus is large. Some large nuclei showed several nucleoli. The celluler polymorphism is very marked and there are many cells with exceedingly large nuclei which are often lobulated. Mitotic figure are present in fair numbers, although frequency varied from tumor to tumor. The No. 5 tumor showed unusually large number, several mitotic figures being seen in a single microscopic field. In rare cases mitotic figures were atypical but multipolar type was not observed. In many cases

round multinucliated giant cells were seen scattered through tumor tissues. The No. 1 tumor showed exceptionally large number of these giant cells.

Vascularity of the tumors varied. Some showed a relatively large area in which numerous large blood spaces were seen. The tendency for haemorrhage was generally slight and infiltration of red blood-cells into the tumor tissue was observed but rarely. Necrosis was found in varying extent in different tumors but was generally slight. Only one tumor (No. 7) showed extensive central necrosis, while others contained a few small necrotic foci.

The general histologic features suggested that some of these sarcomas may be rhabdomyosarcoma at least in part. In single tumor there were usually rhabdomyosarcomatous as well as fibrosarcomatous areas. We have not yet confirmed the diagnosis of rhabdomyosarcoma by demonstrating striated muscle fibrils, and for this purpose minute cytological studies are needed. Further studies along this line should also include observations on histogenesis. However, it is sufficient to state, for the purpose of the present report, that all the tumors produced in this experiment were malignant mesoblastic neoplasms.

Discussion

The first question that arises in the above experiment is whether propylene glycol is in itself carcinogenic. In unpublished experiment, I injected xanthene in propylene glycol one or two times a month subcutaneously at the same site of 12 rats, with which experiment was started. 9 rats survived over 302 days, receiving xanthene 110 mg, and propylene glycol 11 cc in 11 injections. No notable local tissue change, to say nothing of tumor, was found in any of the 9 rats. Although the experimental conditions here were not exsactly the same as in m-toluylenediamine experiment discribed in this paper, the entirely negative results may be taken to speak for the lack of carcinogenisity in propylene glycol. To make this point quite sure an experiment is now being carried out using propylene glycol alone.

Another question is whether m-toluylenediamine in waterly solution is really incapable of sarcoma production. Nagata's negative results already referred to are not conclusive, since his experiment was terminated in 41 days, a period altogether too short for the experiment of this nature. In the face of these facts it may be premature to definitely assign a decisive role to propylene glycol as solvent, but fact remains that sarcoma was produced in 100% of rats by injection of propylene glycol solution of m-toluylenediamine, carcinogenecity of which has not hitherto been demonstrated.

In this connection attention may be called to more or less similar experiments in which sarcoma was produced in rats by injections of concentrated solution of glucose (27) and other sugars (28), implantation of cellophane (29), etc. In prac-

tically all these cases sarcomas were produced only in a small percent of the rats used. The consistently small percent of sarcoma produced in these experiments seemed as though it was a universal rule for experiments of this type. This apparent rule has now been broken by the very high rate of sarcoma production by injections of propylene glycol solutions of m-toluylenediamine, which would mean either that m-toluylenediamine is in fact a carcinogen of the order of methylcholanthrene and related hydrocarbons, or that propylene glycol is capable of greatly magnifying the naturally weak carcinogenic action of m-toluylenediamine.

If the use of propylene glycol as solvent can be proved to be responsible for strongly bringing out the otherwise feeble carcinogenic action of m-toluylenediamine, interesting problem will arise as to the mechanism involved in this enhancing action. It seems possible that propylene glycol may facilitate the penetration of m-toluylenediamine into the cells, but further studies are needed to discuss this point.

The practical implication of the possible carcinogenesis-enhancing action of propylene glycol in human hygiene may perhaps be worth calling attention too, in view of the present wide use of this substance as an ingredient in various cosmetic preparations.

Summary

Sarcoma was produced by repeatedly injecting 0.4% solution in propylene glycol of m-toluylenediamine subcutaneously into normal rats. Injections were made in doses of 0.5 cc at as nearly the same site as possible on the back, and at the rate of once a week for about 8 months, and sarcoma developed in all the 9 rats that survived this period. No notable change in internal organs, no liver cirrhosis in special, was found in these rats.

The possibility of propylene glycol having enhanced the carcinogenic activity of m-toluylenediamine was discussed.

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Explanation of Figures

Plate XXIII

Fig. 1. Rat No. 1, receiving 60 mg of m-toluylendiamine in 29 injections, 297 days after the first injection and 44 days after discontinuing the injection.

Fig. 2. Rat No. 2, receiving 72 mg of m-toluylenediamine in 35 injections, 348 days after the first injection and 27 days after discontinuing the injection.

Fig. 3. Rat No. 5, receiving 76 mg of m-toluylenediamine in 37 injections, 384 days after the first injection and 32 days after discontinuing the injection.

Plate XXIV

Fig. 4. A typical fibrosarcomatous picture of the tumor produced by the injections of m-toluylenediamine in propylene glycol.

Fig. 5. The same in higher magnification, showing mitotic figures.

Fig. 6. A somewhat more rhabdomyosarcomatous area of the tumor, with large, multinucleated cells.

Fig. 7. The same, showing invasion of muscular tissue by the sarcoma.

要　旨

m-Toluylenediamine による肉腫の実験的成生

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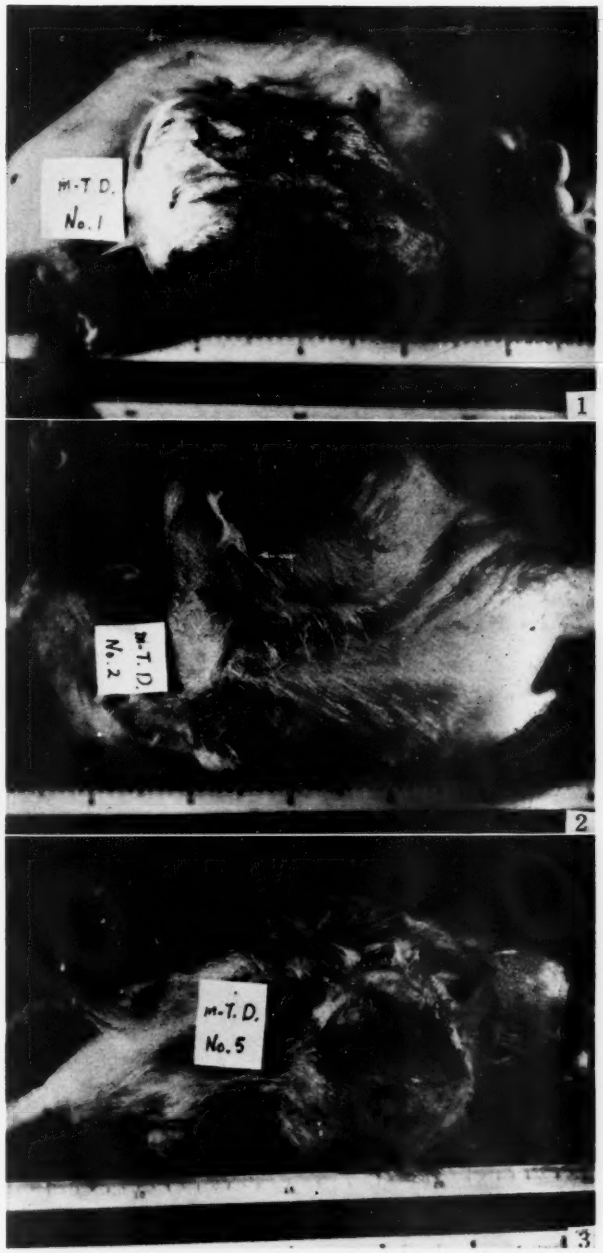
現在まで非発癌物質とされていた m-Toluylenediamine の 0.4% Propylene Glycol Solution を白鼠の皮下に反復注射することによって (注射量・1 週間 1 回, 0.5 cc 宛), 8 カ月以上生存した 9 匹の白鼠のうちの 9 匹 (発癌率・100%) に肉腫を発生せしめた。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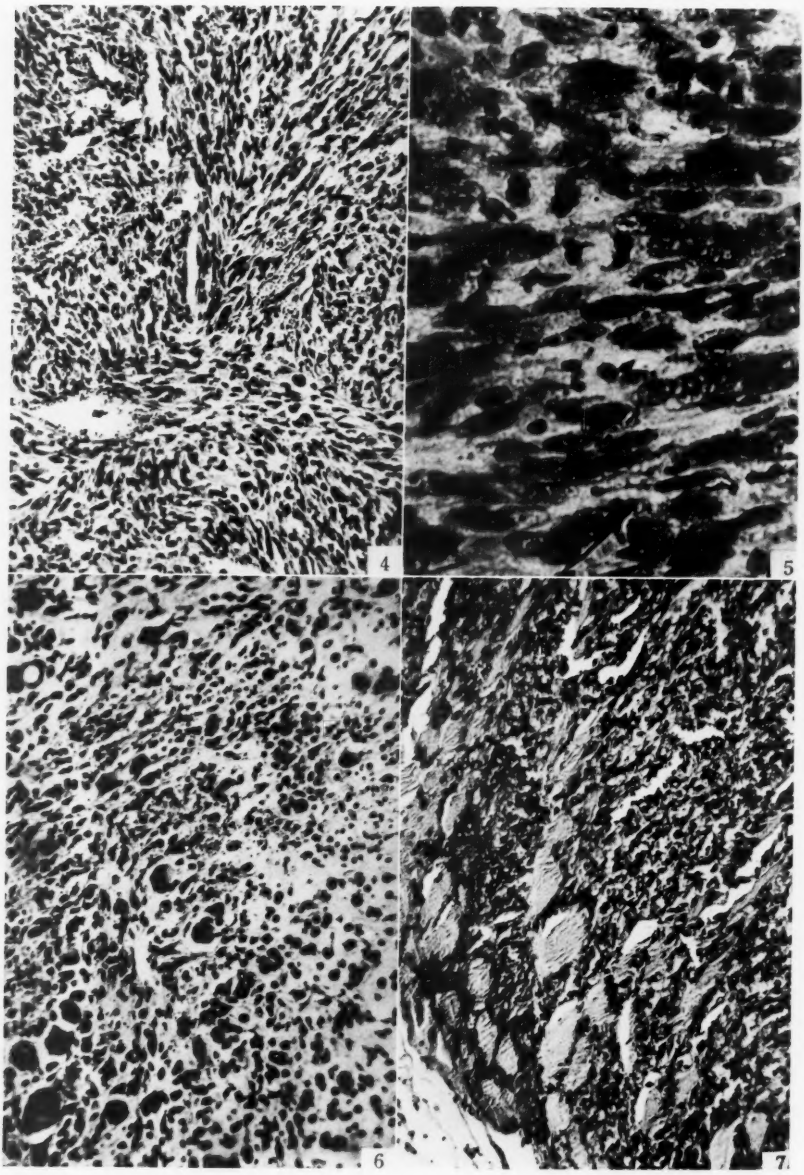
1
m-T.D.
No. 2
2
m-T.D.
No. 5
3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[GANN, Vol. 46; December, 1955]

ON THE ANTI-CANCER ACTION OF QUINOLINE DERIVATIVES

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In a previous report we (1) described the results of screening test on the anti-cancer action of quinone derivatives, based on the *in vitro* method, using NF mouse sarcoma, deviced by Fukuoka (2). Having found that this simple method is very dependable we have now extended our screening tests to quinoline derivatives. In these tests *in vivo* effect on Ehrlich carcinoma, using both ascites and solid forms, was also investigated, and correlation between the *in vitro* and *in vivo* effects was noted.

A considerable amount of literature exists as to the anti-cancer action of quinoline derivatives, but with few exceptions, previously tested compounds showed no notable action (3); Badger et al. (4) and Haddow et al. (5) worked on styrylquinoline, and Lewis et al. (6) on quinoline dyes. More recently von Euler and Hasselquist (7) reported a marked inhibiting effect of 6-aminoquinaldine-4-carbonic acid on the proliferation of the Yoshida ascites sarcoma. Hughes et al. (8) claimed that transplanted rat lymphoma can be made to disappear by the oral administration of 2- or 4-(-p-dimethylaminostyryl)quinoline methiodine or ethiodide.

We have synthesized a large variety of quinoline derivatives and by testing them, found that 4-nitroquinoline-N-oxide and some of its derivatives show a marked tumoricidal action. This group of quinoline compounds was first synthesized by Ochiai et al. (9) by preparing N-oxide of quinoline by means of hydrogen peroxide and then by adding nitro radical. The anti-cancer actions of this type of derivatives have not hitherto been investigated, so far as we are aware.

Experimental Methods

In Vitro Tests. Substances were tested at the concentrations of 0.05 percent, 0.01 percent, and 0.005 percent in physiological salt solution, adjusting the reaction to pH 6.0–7.0 by adding sodium bicarbonate when necessary. As control, physiological salt solution of pH 7.0 was used. The tumor used was 10 to 14 day old well growing transplantable NF mouse sarcoma. Approximately the same amount of the NF sarcoma tissue, cut up into fragments of about 1 mm diameter, was

immersed in these solutions in small Petri dishes, and was allowed to stand at 4–7°C for 24 hours, after which the sarcoma tissue fragments from the four dishes (containing three different concentrations of the test substance and blank control) were implanted into the subcutaneous tissue at four different sites of one mouse. In every test, at least three mice were used, and the growth of tumors resulting from the implantation was observed for two weeks or more. When control graft did not take, the case was discarded as that of natural immunity.

In Vivo Tests. 1). Ehrlich ascites carcinoma: Freshly aspirated ascites fluid of Ehrlich mouse carcinoma, one week after the inoculation, was injected in 0.2 cc doses intraperitoneally into groups of usually five normal mice each of about the same body weight. The ascitic fluid injected contained about 1,000,000–1,500,000 cells per cc, i. e., about 200,000–300,000 cells in 0.2 cc. Treatment was started 48 hours after the inoculation of ascitic fluid and was continued for 5 days. The treatment consisted of daily injection into the peritoneal cavity of the substance to be tested. When the substance was insoluble in water, the suspension was stabilized by adding sufficient amount of finely pulverized carboxymethyl cellulose in the salt solution. Weekly body weight changes and the number of days of survival were used as criteria in judging the effect of tested substance, always with due attention to the state of abdominal distension and general physical condition of the mice. Microscopical examination of ascites was made only when necessary in order to establish the diagnosis.

2) Ehrlich Carcinoma (solid form): 0.2 cc of 10 day old Ehrlich carcinoma ascites containing 150,000–500,000 cells, was injected subcutaneously at two different sites, taking usually 5 normal mice for a group. 24 hours later treatment was started by injecting intraperitoneally 0.25 cc of the solution of the substance to be tested, injections being repeated twice daily for 10 days. On the 11 th day the mice were killed and body weight change and tumor weight were determined and compared with those in the untreated control group.

Toxicity Tests. Toxicity of the substances was tested by injecting intraperitoneally 0.5 cc of the suspension of the fine powder in 1 percent solution of carboxymethyl cellulose in a single dose. Normal mice of 15 g body weight were used. Number of deaths was noted daily for one week, and LD_{50} for each day was calcurated according to the method of Bährens and Körber.

Results

Tables 1 to 4 show the details of *in vitro* tests. In these tables the results are expressed in the term of the number of the negatives over the total number of implants made for each dilution of test substance. For example, 3/3 indicates that all the three implants resulted in no tumor growth: 1/3, only one out of three implants resulted in negative, that is, two tumors produced from three implants.

The manus sign (−) means that all the implants resulted in tumor production, that is, no effect. Cases in which there were only very small tumors compared to the control group are indicated by the ± sign.

Results of *in vivo* tests are shown in Tables 5-7. These tables require no explanation. The survival days in parenthesis in the table refer to the case of death while the animal was still under treatment, and are not included in the calcuration of the average survival days for the group.

Table 8 contains the details of the toxicity tests on some selected derivatives. The results are expressed in the term of number of deaths over the total number of the mice used, every day for 7 days, at different dosage levels.

Discussion of the Results

In discussing *the results of the in vitro tests using NF sarcoma*, it is convenient to classify the quinoline derivatives tested into the following four groups—:

1). The group of compounds in which the nitrogen of the quinoline nucleus is of the oxide type, without nitro radical. (Table 1).

2). Group of compounds in which the quinoline nucleus has nitro radical, and the nitrogen is not of the oxide type. (Table 2).

3). Group of compounds in which the quinoline nucleus has nitro radical at the position 4, and the nitrogen is of the oxide type. (Table 3).

4). Group of compounds in which the quinoline nucleus has no nitro radical, and the nitrogen not in oxide type. (Table 4).

The first group includes 8 kinds of quinoline-N-oxide derivatives. As may be clear from Table 1, none of these derivatives showed a recognizable tumoricidal activity.

In the second group, there are 9 kinds of quinoline derivatives with nitro radical.

Table 1. Effect of Quinoline-N-oxide Derivatives on the survival *in vitro* of NF mouse sarcoma. Group 1.

Compounds			Tumoricidal effect at			
No.		M. P.	0.05%	0.01%	0.005%	0.002%
1935	Quinoline-N-oxide dihydrate	61	—	—	—	—
2055	Quinaldine-N-oxide	77–82	—	—	—	—
2061	6-Methylquinoline-N-oxide	48–50	—	—	—	—
2124	3-Methylquinoline-N-oxide	37–39	—	—	—	—
2144	7-Chloroquinoline-N-oxide	74–78	—	—	—	—
2067	6-Bromoquinoline-N-oxide	165–170	—	—	—	—
2051	4-Aminoquinoline-N-oxide hydrochloride	271–274	—	—	—	—
1938	4-Thioglycolylquinoline-N-oxide	203–204	—	—	—	—

Table 2. Effect of Nitroquinoline Derivatives on the survival *in vitro* of NF mouse sarcoma. Group 2.

Compounds			Tumoricidal effect at			
No.		M. P.	0.05%	0.01%	0.005%	0.002%
2052	4-Nitroquinoline	87–89	—	—	—	—
2030	6-Nitroquinoline	146–148	—	—	—	—
2031	6-Methoxy-8-nitroquinoline	158–160	—	—	—	—
2080	6-Bromo-5-nitroquinoline	128–130	—	—	—	—
2045	8-Ethoxy-5, 7-dinitroquinoline	275	3/3	1/3	—	—
2020	5-Nitroquinaldinic acid	201–203	—	—	—	—
2022	2-(4'-nitrophenyl)-4-carboxyquinoline	222–225	3/3	—	—	—
2023	8-Nitroquinaldinic acid	175–177	—	—	—	—
2046	8-Ethoxy-5-nitroquinoline	125–128	—	—	—	—

Of these, only two showed weak tumoricidal activity at the dilution of 0.05 %. (Table 2).

The third group consists of the derivatives of the general structure of 4-nitroquinoline-N-oxide (1936). In this group, 4-nitroquinoline-N-oxide (1936), 4-nitroquinaldine-N-oxide, (2054) or 4-nitro-2-alkylquinoline-N-oxide, 4-nitro-2-ethylquinoline-N-oxide (2126), and 4-nitro-2-n-propyl quinoline-N-oxide (2138) manifested distinct tumoricidal action at the high dilution of 0.002%, 0.002%, 0.001%, and 0.001%, respectively. 6-Bromo-4-nitroquinoline-N-oxide also inhibited the proliferation of NF sarcoma, although it did not show complete tumoricidal effect at 0.01% or 0.005% dilutions. This fact probably means that the substance does not dissolve well at the low temperature of the *in vitro* tests (4–7°C), and that the

Table 3. Effect of 4-nitroquinoline-N-oxide Derivatives on the survival *in vitro* of NF mouse sarcoma. Group 3.

Compounds			Tumoricidal effect at					
No.		M. P.	0.05%	0.01%	0.005%	0.002%	0.001%	0.0005%
1936	4-Nitroquinoline-N-oxide	151–153	4/6	6/6	4/6	3/3	±	—
2054	4-Nitroquinaldine-N-oxide	153–157	4/4	4/4	4/4	3/3	±	—
2126	4-Nitro-2-ethylquinoline-N-oxide	144–146	3/3	3/3	3/3	3/3	3/3	—
2138	4-Nitro-2-n-propylquinoline-N-oxide	156–160	2/2	2/2	3/3		3/3	±
2068	6-Bromo-4-nitroquinoline-N-oxide	205–207	1/3	±	±			
2062	6-Methyl-4-nitroquinoline-N-oxide	182–183	1/6	—	—			
2079	6-Bromo-4, 5-dinitroquinoline-N-oxide	233–235	—	—	—			
2056	8, 4-Dinitroquinoline-N-oxide	213–214	—	—	—			

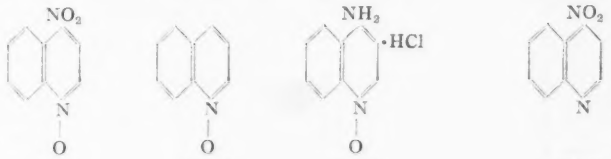
Table 4. Effect of Quinoline Derivatives on the survival *in vitro* of NF mouse sarcoma. Group 4.

Compounds			Tumoricidal effect at			
No.		M.P. or B.P.	0.05%	0.01%	0.005%	0.002%
25	8-Hydroxyquinoline	73-75	3/3	—	—	
2043	8-Ethoxyquinoline	B.P. 282-286	—	—	—	
2058	6-Methylquinoline	B.P. 256	3/3	—	—	
1874	2, 4, 6-Trimethylquinoline	276-278	3/3	—	—	
2100	Quinaldine-β-carboyethyl ester	67-69	3/3	—	—	
2106	2-(β-diethylaminoethyl)quinoline	B.P. 181(12)	3/3	1/3	—	
2120	2-Ethyl-3-methylquinoline	56	3/3	—	—	
2128	2-(p-Dimethylaminostyryl)quinoline	173-175	—	—	—	
1870	2-Phenyl-4-carboxyquinoline	205-207	—	—	—	
1196	Cinchonidine hydrochloride		—	—	—	
40	Quinine dihydrochloride		3/3	—	—	
2019	Quinaldinic acid	154-156	—	—	—	
2015	2-Carbonylaminoquinoline	121-123	—	—	—	
2017	2-Cyanoquinoline	93	—	—	—	
2145	1-Benzoyl-2-cyano-2-hydroquinoline	152-154	—	—	—	
2118	2-Aminoquinoline	125-128	3/3	3/3	2/3	—
2131	2-Mercaptoquinoline	174	3/3	—	—	
2134	2-Methylmercaptoquinoline	53-55	3/3	—	—	
2148	6-Chloroquinoline	38-40	3/3	—	—	
609	3-Cyano-4-carboxyquinaldine	B.P. 242	—	—	—	

actually available concentration was considerably lower than indicated.

Of 20 derivatives of the fourth group, 2-aminoquinoline (2118) alone showed tumoricidal action at the dilution of 0.005%, the rest being inactive.

From the above results it is to be noted that the series of the quinoline derivatives with the oxide type of nitrogen and nito radical at the position 4, and those with alkyl radical at the position 2, manifest a strong tumoricidal action. It is interesting to observe that derivatives with allied structures, such as 4-amino-quinoline-N-oxide hydrochloride (2051), quinoline-N-oxide (1935) and 4-nitroquinoline (2052) are entirely devoid of tumoricidal action.



(1936) (1935) (2051) (2052)

We now turn to the consideration of *the results of in vivo experiments using the ascites form of the Ehrlich mouse carcinoma.* (Tables 5 and 6).

Table 5. Effect of Selected Quinoline Derivatives on Ehrlich Ascites Carcinoma *In Vivo*.

No.	Compounds	Treated Groups				Control Groups			Difference in survival days	Lowest effective concent. *in vitro* test
		Doses mg/kg	No. of mice	Average body wt. change after 1 week	Average survival days	No. of mice	Average body wt. change after 1 week	Average survival days		
1936	4-Nitroquinoline-N-oxide	10	5	−0.88	32.6	5	+1.4	11.2	+21.4	0.002
		7	5	−0.44	44.8	5	+2.2	16.5	+28.3	
2054	4-Nitroquinaldine-N-oxide	8	4	+0.6	18.7	4	+2.2	13.0	+5.7	0.002
		5	4	+0.02	24.0	4	+2.2	13.0	+11.0	
		2	4	+1.4	27.0	4	+2.4	11.2	+15.8	
2126	4-Nitro-2-ethylquinoline-N-oxide	8	5	+0.88	50.2	5	+1.96	14.4	+35.8	0.001
		5	4	−1.2	13.5	4	+2.8	14.0	−0.5	
		3	4	+0.3	13.7	4	+4.2	12.7	+1.0	
2138	4-Nitro-2-n-propylquinoline-N-oxide	10	5	−1.4	31.2	5	+2.54	14.0	+17.2	0.001
		3	5	+1.04	16.4	5	+2.54	14.0	+2.4	
2068	6-Bromo-4-nitroquinoline-N-oxide	5	4	−1.2	16.0	4	+2.4	11.2	+4.8	0.05
		3	3	−2.16	40.7	4	+2.85	14.0	+26.7	
		1	4	+0.9	16.5	4	+4.3	12.7	+3.8	
2062	6-Methyl-4-nitroquinoline-N-oxide	15	4	+3.6	15.0	5	+1.96	14.4	+0.6	>0.05
		7	4	+3.8	18.7	4	+2.4	11.2	+7.5	
2052	4-Nitroquinoline	7	4	+1.3	11.5	4	+2.5	13.7	−2.2	>0.05
2118	2-Aminoquinoline	10	4	+1.8	10.0	4	+4.3	12.7	−2.7	0.05
		3	4	+2.4	10.5	4	+4.3	12.7	−2.2	
2100	Quinaldine-β-carboxyethyl ester	10	5	+2.88	1.78	5	+1.96	14.4	+3.4	0.05
		20	4	+2.3	13.0	4	+4.3	12.7	+0.3	
	Methyl-bis(β-chloroethyl)amine-N-oxide. HCl	10	5	+1.3	20.4	5	+1.4	11.2	+9.2	
		5	5	+1.76	16.4	5	+2.54	14.0	+2.4	

Teble 6. Effect of Selected 4-Nitroquinoline-N-oxide Derivatives on Ehrlich Ascites Carcinoma *In Vivo*.

No.	Compounds	Treated Groups								Control Groups				
		Doses mg/kg/day	Mouse No.	Initial body weight g	Body weight after 1 week	Difference	Body weight ater 2 week	Difference	Survival days	Mouse No.	Initial body weight g	Body weight after 1 week	Difference	Survival days
1936	4-Nitroquinoline-N-oxide	7	1	15	13.5	−1.5	14.0	−1.0	93	1	14			(6)
			2	15	15.0	0	14.8	−0.2	46	2	14	17.7	+3.7	17
			3	15	15.7	+0.7	20.2	+5.2	20	3	14	16.2	+2.2	16
			4	15	13.5	−1.5	14.4	−0.6	38	4	14	13.5	−0.5	16
			5	15	14.5	−0.5	15.5	+0.5	27	5	14	17.5	+3.5	17
	(Average)			15	14.4	−0.56	15.7	+0.78	44.8		14	16.2	+2.2	16.5
2068	6-Bromo-4-nitroquinoline-N-oxide	3	1	20.5					(4)	1	17	20.5	+3.5	13
			2	20.5	16.8	−3.7	18.5	−2.0	49	2	17	19.4	+2.4	11
			3	20.5	18.9	−2.5	15.3	−5.2	23	3	17	21.5	+4.5	13
			4	20.5	19.7	−0.8	21.0	+0.5	50	4	17	18.0	+1.0	19
	(Average)			20.5	18.1	−2.16	18.2	−2.2	40.6		17	19.8	+2.8	14
2126	2-Ethyl-4-nitroquinoline-N-oxide	8	1	17.0	18.0	+1.0	17.2	+0.2	99	1	16	16.8	+0.8	12
			2	17.0	16.5	−0.5	11.7	−5.3	14	2	16	20.5	+4.5	8
			3	17.0	18.4	+1.4	18.4	+1.4	53	3	16	16.5	+0.5	11
			4	17.0	16.0	−1.0	15.5	−1.5	50	4	16	17.5	+1.5	26
			5	17.0	20.5	+3.5	21.4	+4.4	35	5	16	18.5	+2.5	15
	(Average)			17.0	17.9	+0.88	16.8	−0.1	50.2		16	17.9	+1.96	14.4

4-Nitroquinoline-N-oxide (1936) prolonged the survival of mice by 21.4 and 28.3 days on averages over the controls by the administration of 10 mg per kg of body weight per day and of 7 mg per kg respectively. The therapeutic effect of this substance may be regarded as superior to that of methyl-bis(β-chloroethyl)-amino-N-oxide, as, in a similar experiment, this latter substance increased the average survival period over the controls only by 9.2 days. In experiments with this quinoline derivative, 3 of the 5 mice used showed the complete disappearance of tumor cells in the peritoneal cavity, and death was due to the growth of solid tumors inadvertently produced in the subcutaneous tissue.

4-Nitroquinaldine-N-oxide, containing alkyl radical in the position 2 (2054) prolonged the survival over controls by 15.8 days on an average in doses ot 2 mg per kg per day. 4-Nitro-2-ethylquinoline-N-oxide (2126) prolonged the survival by 35.8 days in 8 mg per kg per day doses.

6-Bromo-4-nitroquinoline-N-oxide (2068), in doses of 3 mg per kg per day, prolonged the survival by 26.7 days. In this case there was some slight decrease in body weight, but in 2 of the 4 mice in the experiment tumor cells in the peritoneal fluid were totally destroyed and the death of the mice was caused by the growth of the incidental subcutaneous solid tumors.

At this point, we would call special attention to the fact that the all the above mentioned derivatives, proved to be active in the therapy of Ehrlich ascites carcinoma *in vivo*, are those that showed strong tumoricidal action at high dilutions in *in vitro* tests. Also, 4-nitroquinoline (2052), 2-aminoquinoline (2118), and quinaldine-β-carboxyethyl ester (2100), none of which showed any effect on Ehrlich ascites carcinoma *in vivo*, are the ones that manifested either no tumoricidal action *in vitro* on NF sarcoma or only at a very low dilution.

We next consider *the effect on the growth of solid form of the Ehrlich carcinoma subcutaneously transplanted.* The four quinoline derivatives which showed marked effect on the ascites form come under this head. (Table 7). With the exception of the case of methyl-bis(β-chloroethyl)amine-N-oxide, all the experiments were carried out at the same time in order to make the comparison easier. Doses administered were generally 2—3 times larger than those that were found to be optimum for the treatment of the ascites form.

4-Nitroquinoline-N-oxide (1936) showed the tumor inhibition ratio of 67% in the doses of 15 mg per kg per day, which is quite comparable with the effect of methyl-bis(β-chloroethyl)amine-N-oxide, which produced a similar inhibition ratio in the doses of 20 mg per kg per day. In the case of this latter substance, however, there was a marked reduction of body weight of the mice, indicating considerable toxic effect, and it seems necessary to take into consideration the reduced tumor growth due to this unfavorable physical condition on the part of the host. When the doses of methyl-bis(β-chloroethyl)amine-N-oxide was

Table 7. Effect of Selected 4-Nitroquinoline-N-oxide Derivatives on Ehrlich Carcinoma (Solid) *In Vivo*.

No.	Compounds	Doses mg/kg/day	No. of deaths after 11 days	Av. body wt. change after 11 days Treated/Controls	Av. wt. of tumor after 11 days Treated/control (A)	Tumor inhibition ratio (1-A)×100
1936	4-Nitroquinoline-N-oxide	15	0/5	−0.44 / +4.1	0.6 / 1.18	67
2054	4-Nitroquinaldine-N-oxide	10	0/5	−0.06 / +4.1	0.82 / 1.81	55
2126	2-Ethyl-4-Nitroquinoline-N-oxide	7	0/5	+2.6 / +4.1	1.07 / 1.81	41
2068	6-Bromo-4-Nitroquinoline-N-oxide	5	0/5	+2.96 / +4.1	1.08 / 1.81	41
	〃	8	2/5	+0.7 / +4.1	0.81 / 1.81	56
	Methyl-bis(β-chloroethyl)amine-N-oxide hydrochloride	5	0/5	+0.4 / +2.66	1.22 / 1.52	20
	〃	20	0/5	−2.2 / +4.1	0.651 / 1.81	66

Table 8. Results of Toxicity Tests

Compound	Hours	20 mg / kg	40 mg / kg	60 mg / kg	80 mg / kg	100mg / kg		LD_{50} per kg
1936	24	0/4	0/4	2/4	2/4	4/4		70mg
4-Nitroquinoline-	48	0/4	0/4	3/4	2/4			65mg
N-oxide	72	0/4	1/4	3/4	4/4			50mg
	96	0/4	2/4	3/4				45mg
	120	0/4	2/4	4/4				40mg
	144	0/4	4/4					30mg
		20 mg / kg	40 mg / kg	60 mg / kg	80 mg / kg	100mg / kg		
2054	24	0/4	0/4	0/4	0/4	0/4		over 100mg
4-Nitroquinaldine-	48	0/4	0/4	0/4	0/4	0/4		〃
N-oxide	72	0/4	0/4	0/4	0/4	3/4		
	96	0/4	0/4	2/4	1/4	3/4		
	120	0/4	1/4	2/4	2/4	4/4		60mg
	144	0/4	1/4	2/4	3/4			60mg
		5mg / kg	10 mg / kg	20 mg / kg	40 mg / kg	60 mg / kg		
2068	24	0/4	0/4	0/4	0/4	0/4		over 60mg
6-Bromo-4-Nitro-	48	0/4	0/4	0/4	1/4	0/4		
quinoline-N-	72	0/4	0/4	0/4	2/4	2/4		
oxide	96	0/4	0/4	1/4	4/4	3/4		26.25mg
	120	0/4	0/4	2/4		4/4		22.5 mg
	144	0/4	0/4	3/4				18.75mg
		10 mg / kg	20 mg / kg	40 mg / kg	60 mg / kg	80 mg / kg	100mg / kg	
2126	24	0/4	0/4	0/4	0/4	0/4	0/4	over 100mg
4-Nitro-2-ethyl-	48	0/4	0/4	0/4	0/4	0/4	1/4	
quinoline-N-oxide	72	0/4	0/4	0/4	0/4	0/4	2/4	
	96	0/4	0/4	0/4	0/4	2/4	4/4	80mg
	120	0/4	0/4	0/4	0/4	3/4		75mg
	144	0/4	0/4	0/4	1/4	3/4		70mg
		10 mg / kg	20 mg / kg	40 mg / kg	60 mg / kg	80 mg / kg	100mg / kg	
2138	24	0/4	0/4	0/4	0/4	0/4	0/4	over 100mg
4-Nitro-2-n-propyl-	48	0/4	0/4	0/4	0/4	1/4	1/4	
quinoline-N-oxide	72	0/4	0/4	0/4	0/4	1/4	4/4	85mg
	96	0/4	0/4	0/4	0/4	1/4		〃
	120	0/4	0/4	0/4	0/4	1/4		〃
	144	0/4	0/4	0/4	0/4	2/4		80mg

reduced to 5 mg per kg, which showed no toxic effect, the tumor inhibition ratio was also reduced to 20%. In contrast to this, 4-nitroquinoline-N-oxide (1936), in the optimum doses, produced merely negligible effect on the body weight.

4-Nitroquinaldine-N-oxide (2054) may be said to have about the same degree of solid tumor inhibiting action as 4-nitroquinoline-N-oxide, to judge from the actual inhibition ratio and body weight changes observed.

The same may also be said of 6-bromo-4-nitroquinoline-N-oxide (2068).

Finally, as regards *the toxicity*, it may be noted that LD_{50} as determined in the usual way, changes according to the time observed after the injection. This characteristic manifestation of the toxicity is conceivably related to the solubility of these substances. In Table 8 are given data for LD_{50} for the period of 7 days.

Summary

A large series of quinoline derivatives were screened as to their anti-cancer action by means of *in vitro* test, using NF mouse sarcoma, and of *in vivo* tests, using both ascites and solid forms of Ehrlich mouse carcinoma. As the result of these tests, it was discovered that 4-nitroquinoline-N-oxide, 4-nitro-2-alkyl-quinoline-N-oxide, and 6-bromo-4-nitroquinoline-N-oxide show a very marked anti-cancer activity.

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要　旨

キノリン類の制癌作用

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われわれは NF 肉腫を用うる in vitro の方法で多数のキノリン誘導体の制癌作用を検して 4-nitroquinoline-N-oxide, 4-nitro-2-alkylquinoline-N-oxide および 6-bromo-4-nitroquinoline-N-oxide が極めて強い制癌作用を有することを発見した。更に Ehrlich 腹水癌を使用して延命効果を観察するに 4-nitroquinoline-N-oxide は 7 mg/kg で 6-bromo-4-nitroquinoline-N-oxide は 3 mg/kg で 2-ethyl-4-nitroquinoline-N-oxide は 8 mg/kg でそれぞれ対照より 28.3 日，26.7 日 および 35.8 日 延命している。Ehrlich 癌 (固型) に対しても之等化合物は対照に比して相当発育を抑制している。

(一部分文部省科学研究費による)

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PREPARATION OF POTENT CONCENTRATES OF TOXOHORMONE FREE FROM NUCLEIC ACID

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Since Nakahara and Fukuoka (1948) (1, 2) succeeded in extracting a liver catalase depressing factor, the so-called toxohormone, from malignant tumor tissue, their procedure has been applied by Greenfield et al. (3) and other workers in many laboratories, and proved of great value in all cases. It consists of boilling water extraction followed by alcohol fractionation, and yields amorphous powder active for normal mice in 50–100 mg doses. Further purification is attained by several means, that is, acid or cupric sulfate precipitation. By these treatments, toxohormone concentrates, which are active in 5–10 mg dose, have been obtained.

As remarked by Endo (4) in this laboratory, the above described procedures are very similar to nucleoprotein preparation method, and actually it was demonstrated that some toxohormone preparations from different origins contain nucleic acid as much as about 30 % of their weight. But nucleic acid itself obtained from rhodamine sarcoma (Umeda) by Clark-Schryver's (5) method showed no activity as toxohormone, and so it was concluded that nucleic acid might not be the active principle of toxohormone. As regards the chemical nature of toxohormone, the early experiments of Nakahara and Fukuoka (1, 2) indicated already that it may be a kind of proteose or polypeptide. Their recent study (6) about the biosynthesis of toxohormone from amino acids clearly supports the polypeptide nature of its active principle. Moreover, from the fact that nucleic acid is strongly coherent in all samples, one may assume that the polypeptide in toxohormone should be basic in nature.

It was, therefore, decided in this study to extract a basic polypeptide from tumor tissues and examine its toxohormone activity. For this purpose we adopted the procedure of the Merck Research Laboratory colleagues[7], who have succeeded in preparation of corticotropin from whole hog pituitary glands in good yields. Their first process consists of acetone defatting, extraction with methanol-acetic acid mixture, and then precipitation with ether. Appling the same procedure on several tumor tissues, we obtained a white amorphous powder which was active as toxohormone in 20–40 mg doses, and fortunately free from nucleic acid contamination.

Although the above described preparation method gives samples of considerable high potency, in good yield, the resulting samples show a toxicity which cannot be overlooked. It has been demonstrated by Nakahara and Fukuoka that raw "toxohormone" is fairly devoid of toxicity, and therefore it was hoped that a satisfactory samples of no toxicity and of no contamination with nucleic acid, may be obtained by applying acid-methanol extraction to raw "toxohormone". Our further experiments clearly demonstrated that this is possible, and we could obtain a sample which was active in 10 mg doses and free of nucleic acid.

The details of the procedure and the relation between new preparation and original preparation will be described in this paper.

Experimental

The following tumors were employed: 1) Umeda rhodamine sarcoma (rat), 2) NF sarcoma (mouse), 3) transplantable hepatoma (rat). All of them were carefully dissected free of necrotic areas, and after weighing frozen by dry ice-acetone.

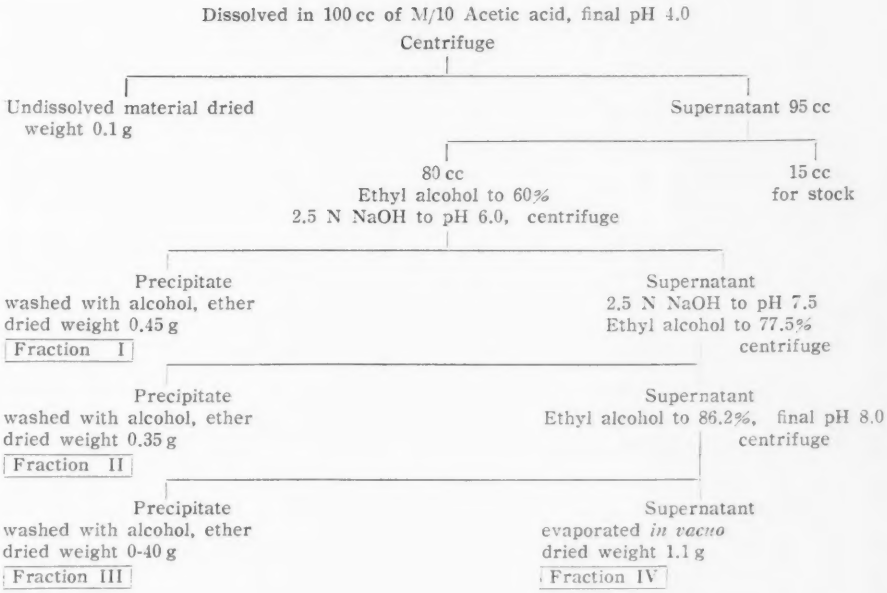
Preparation of Acetone Dry Powder.— Frozen tumor tissue was allowed to thaw partially while covered with acetone, then homogenized with 3 parts (v/w) acetone in Waring blender. The mixture was transfered to a beaker and stirred with additional two parts of acetone for two hours. The mixture was centrifuged and the residue was washed twice more with three parts acetone, then with three parts of methanol. After a final washing with ether, the solid was dried over H_2SO_4 *in vacuo*.

In Preparation No. IV of Table 3, heat dried tissue was defatted with ether extraction by means of Soxhlet's apparatus, and after being dried, used for subsequent extraction.

Extraction of Defatted Tissues with Methanolic Acetic Acid.— Acetone dried tissue was placed in a three-necked flask fitted with mechanical stirrer, reflux condenser open to the atomospher through a sodalime tube, and thermometer. To the flask was added 9 parts of methanol (v/w) and then with stirring 6 parts (v/w) of glacial acetic acid, and the mixture was refluxed (ca. 75°) for two hours. At the end of this time, the contents were allowed to cool to room temperature. The mixture was centrifuged and the brown colored supernatant was collected. The residue was washed with a 40% solution of acetic acid in methanol and the washing were added to the collected decantate. The combined fluid was diluted with an equal volume of ether. At this time a snow white flocculation appeared, which soon settled down to bottom. The precipitate was collected by centrifugation and washed with ether and dried. This fraction was designated as O-Fraction in this paper.

Ethyl Alcohol Fractionation of Acid Methanol Extract (O-Fraction).— Further purification was carried out by means of ethyl alcohol fraction. An

Table 1. Ethyl alcohol Fractionation of O-Fraction
Staring material: Preparation No. V (Table 3) 2.0 g
From rhodamine sarcoma (Umeda)



outline containing the essential features of this procedure is presented in Table 1. The starting material for this experiment was No. V preparation of O-Fraction from rhodamine sarcoma, which was proven to be active in 20 mg dose as demonstrated in Table 3. Because the O-Fraction is rather more soluble in weak acid than in neutral, as being expected from its extraction procedures, 2 g of No. V preparation was dissolved in 100 cc of M/10 acetic acid. The reaction of this solution was pH 4.0 (B. C. G.), and at this acid reaction, addition of ethyl alcohol in far excess caused no precipitation, then alcohol was added as far as 60% (v v) and the mixture was brought to pH 6.0, by the addition of 2.5 N NaOH. After standing 2 hours at room temperature, the flocculent precipitate which had been formed was collected by centrifugation. This was reserved as Fraction I. Fraction II was obtained by bringing the supernatant to pH 7.5, and ethyl alcohol concentration to 77.5%. Subsequently, Fraction III was obtained by raising the alcohol concentration to 86.2%. Further addition of ethyl alcohol and elevation of pH produced no more precipitation, then the supernatant was evaporated *in vacuo* to dryness. The residual white amorphous powder was reserved as Fraction IV. As it was contaminated by sodium acetate, which had been formed during the fractionation, Fraction IV was 1.1 g in weight but only 1/10 of its

Table 2. The Means of Liver Catalase Activity and Thymus Weight of Control Mice.

Experimental group	Number of mice	Mean of catalase activity (0.2 ml.)	Thymus (mg./g. body weight)
1	6	16.45	2.37
2	3	19.03	2.58
3	6	14.84	1.90
4	5	16.64	1.61
5	5	16.50	1.80
6	6	18.45	1.94

Total cases 31 Total mean 16.80 ml.

Table 3. The Effects of O-Fraction of Rhodamine Sarcoma on Liver Catalse Activity and Thymus Weight.

Experimental Group**	Preparation No.	Yield (% of Acetone powder)	Injected dose(mg)	Mice number	Means of Catalase activity (0.2 ml.)	Catalase Decreased%	Mean of Thymus weight (mg./g. body weight)
4	I	4.5	10	6	10.27	39	0.88
4	III	10.0	20	6	8.83	47.5	0.79
5	V	7.85	20	5	8.86	47.3	0.77
6	IV*	5.8	20	6	11.33	32.5	1.21
4	Preparation of Nakahara's method	8.0	70	5	8.2	51.2	1.34
4	Preparation of Nakagawa's method	—	50	3	9.37	44.3	1.04

* Preparation No. IV was prepared from the dried tissue, not from acetone powder.

** The figures of experimental groups are corresponded with that of control groups in table 2.

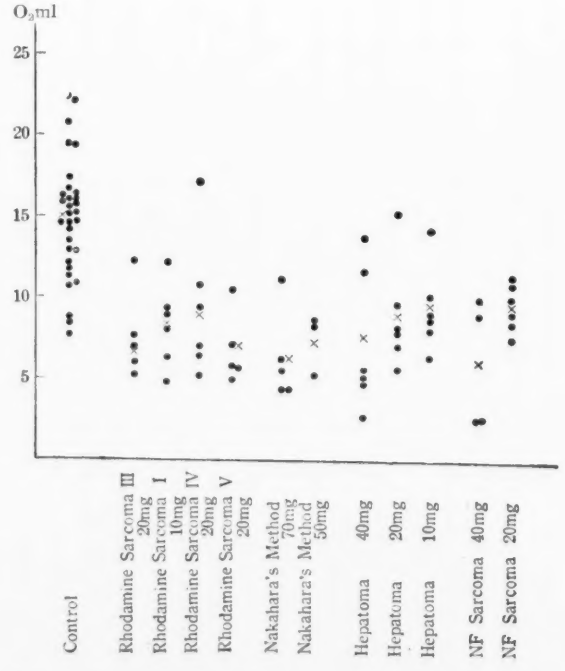


Fig. 1 The Effects of O-Fractions from Different Tumors On Liver Catalase Activity

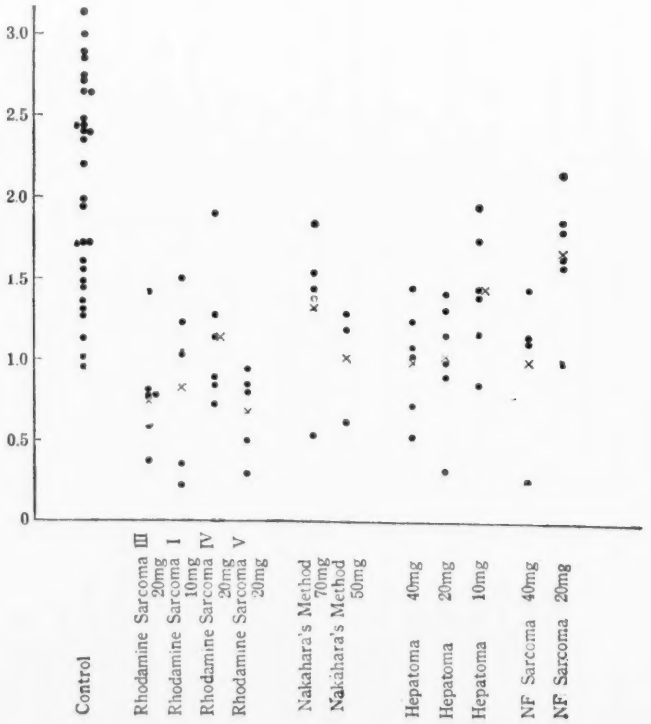


Fig. 2 The Effects of O-Fractions from Different Factors on Thymus Weight mg/g Body Weight

(The figure × in the chart represents the mean of each group)

dried weight was polypeptide as revealed by biuret reaction.

Assay.— The toxohormone activity of the samples to be tested was assayed by the procedures described by Nakahara and Fukuoka (2). Throughout these experiments normal male mice of 15-20 g body weight were employed for this purpose.

Chemical Examination.—Nucleic acid content of each sample was estimated by phosphorus determination (Fiske and Subbarow (14)), and examination of ultravilet absorption.

Polypeptide contents were estimated by biuret reaction (8), and the results were calculated comparing with those of serum globulin which was employed as standard. Because the different proteins give slightly different optical densities by the reaction, there may be some uncertainty in the expression of this results.

Results

Control data: In the course of this experiment, every test was carried out with control group, which consisted of about 6 mice of no treatment. The means of liver catalase activity and thymus weight of each control group were tabulated in Table 2, and the individual data were plotted in Figs. 1 and 2. The means of liver catalase activities of each group were fairly constant, and their total mean resulted as 16.8 ml (O_2 ml.). There were scarcely no case of liver catalase activity lower than 10 ml., and thymus smaller than 1 mg/g body weight. In the course of these experiments, therefore, the sample was assumed to be active, when the mean of liver catalase activity of test group resulted lower than 10 ml. About the thymus involuting action, in the case that the average resulted smaller than 1 mg/g body weight it was defined as active.

The effect of acid-methanol extraction of rhodamine sarcoma (Umeda): The results with the acid-methanol extraction of rhodamine sarcoma are summalized in Table 3, in comparison with that obtained by Nakahara's standard method and Nakagawa's method (9) appling to this tumor. The yields of acid methanol extraction from rhodamine sarcoma varied from 4.5 to 10% (w/w) of acetone dried tissue powder.

As one can see from the figures, each preparation showed definite catalase depressing activity, in 20 mg dose, and it was most remarkable that they were also highly effective in decreasing thymus weight. It was also confirmed that the preparation obtained by Nakahara's standard method, showed only slight effect on thymus weight at 24 hrs. after injection, and this was also the case with acid-methanol extraction preparation No. IV, which was obtained from rhodamine sarcoma dried over water bath.

The effect of acid-methanol extraction from hepatoma and NF sarcoma: One sample from hepatoma and two samples from NF sarcoma were prepared apply-

Table 4. The Effects of O-Fraction of Hepatoma (A) and NF Sarcoma (B) on Liver Catalase Activity and Thymus Weight.

A: Hepatome (rat)

Experimental group	Dose mg.	Number of mice	Mean of Catalase activity O_2 ml.	decreased%	Thymus weight mg./g. body weight
1	20	6	11.7	30.4	1.43
3	20	6	11.05	34.0	1.10
5	20	6	9.7	42.3	1.02

B: NF sarcoma (mouse)

Experimental group	Preparation No.	Yield (% of acetone powder)	Dose mg.	Mouse number	Mean of liver catalase activity O_2 ml.	decreased%	Thymus weight mg./g. body weight
3	I	8.6	20	6	11.7	30.4	1.60
5	II	8.0	40	6	8.15	51.4	1.00

ing the acetic acid-methanol extraction method. The yield from hepatoma was 9% (w/w) and that from NF sarcoma was 8.6 and 8.0% (w/w) respectively. Test showed that these extract were active in 40 mg dose, but not so active in 20 mg dose. And it was demonstrated that they were also effective in reducing thymus weight in 40 mg dose, (Figs. 1 and 2, Table 4, A and B).

The effect of ethyl alcohol-fractionation of acid-methanol extraction from rhodamine sarcoma :

Four subfractions obtained from O-Fraction of rhodamine sarcoma, as described in experimental section, were tested in 20 mg doses at the first trial. As illustrated in Fig. 3, Fractions I and II were almost free of catalase depressing activity, but nevertheless they retained fairly the thymus depressing activity. Fraction IV was highly toxic, and nearly all of the mice injected with this fraction in 10 mg doses died after 24 hours. Only the Fraction III exerted both catalase depressing and thymus involuting action in 20 mg or even in so small as 10 mg doses. As shown in Fig. 3, this fraction was also active, when injected in 2 mg dose three times at 24 hours internal, but at this time no deffinite effect was observed

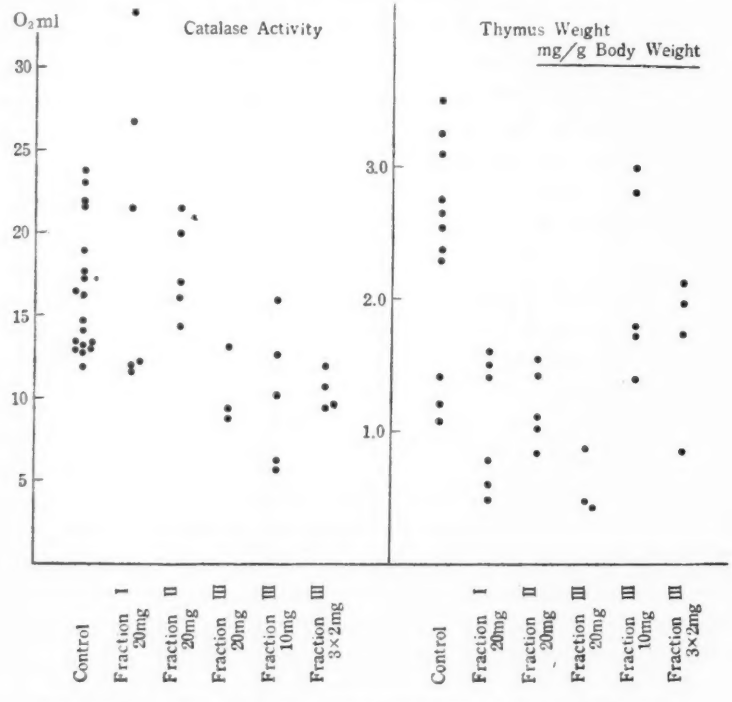


Fig. 3 The Effect of Ethyl Alcohol-Fractionation of Acid-Methanol Extraction from Rhodamine Sarcoma

in thymus weight.

Acid methanol extraction of raw "toxohormone":

The procedure of acid-methanol extraction was applied to raw toxohormone, i. e., that of the stage of alcohol precipitation. Three preparations were obtained from rhodamine sarcoma and their yields were almost the same in each case, being about 20% (w/w) of the starting materials. These preparations were called TO-Fraction; and as showed in Fig. 4, were effective for catalase depressing activity, in 10 mg, but not in 5 mg doses. As the original toxohormone, they showed only slight effect on thymus weight at 24 hrs. after injection.

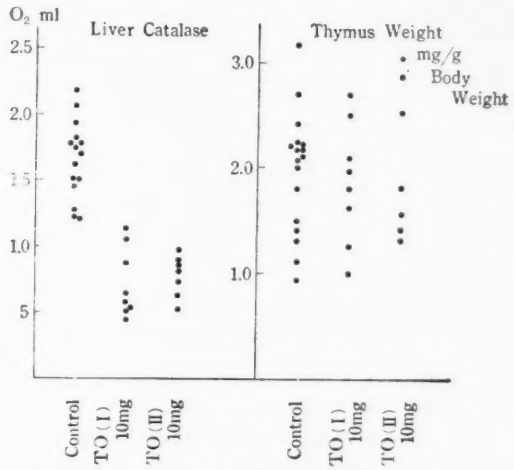


Fig. 4 The Effects of TO-Fractions on Liver Catalase Activity and Thymus Weight

The chemical compositions of the above described preparations:

The polypeptide, nitrogen and phosphorus contents of each preparation are tabulated in Table 5. By biuret reaction, all samples were demonstrated to consist almost entirely of polypeptide. The figures over 100% may have resulted from the inadequacy of serum γ-globulin as standard sample.

Their total phosphorus contents were as much as about 1%, but the fact that all of they were in the form of acid soluble phosphorus, may be enough to show that O-and TO-Fraction were practically free from nucleic acid. Exact determination of inorganic P was interfered by the appearance of turbidity at the addition of molybdate reagent in Fiske-Subbaraw's procedure. But it was estimated that nearly all of the acid soluble P consisted of inorganic P. The presence of TCA soluble (acid soluble) acid molybdate precipitable component in the O-Fraction will be discussed in the following section.

Table 5. Chemical Compositions of O-Fractions

SAMPLE	PEPTIDE %	N %	TOTAL P %	ACID SOLUBLE P / TOTAL P
O-Fraction from Rhodamine sarcoma				
I	103	12.4	1.32	1.00
III	112	14.8	0.78	1.00
IV	91	13.5	0.89	0.95
O-Fraction				
from Hepatoma	110	13.2	0.73	0.95
from NF sarcoma	107	15.2	0.77	1.00
Alcohol Fractiontion				
Fraction III	109		0.92	1.00
TO-Fraction	103		1.50	1.00

Ultraviolet absorption spectrum of each preparation.

For the examination of ultraviolet absorption spectrum, O-Fractions were dissolved in 1/100 m-HCl at a concentration of 0.1%, since they are more soluble in acid medium than in alkaline. The raw toxohormone and its residue after removal of TO-Fraction were extracted by $HClO_4$ under heating and the resulted clear supernatant was used for the spectrophotometric examination. The absorption curves of O-Fractions from rhodamine sarcoma (a), hepatome (b) and NF sarcoma (c), showed very similar aspect with each other (Fig. 5). In contrast with that of raw toxohormone, illustrated in Fig. 6, their absorptions at 220-300 mμ were so low, and do not consist of the typical curves of nucleic acid. They are rather similar to that of protamine, and showed no maximum peak at 280 mμ, which is assumed to be due to tryptaphan and tyrosine (10). This character of the curves is in good accordance with the report that fairly purified toxohormone samples are devoid of tyrosine content.

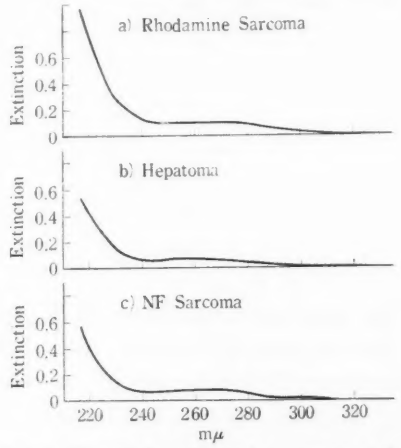
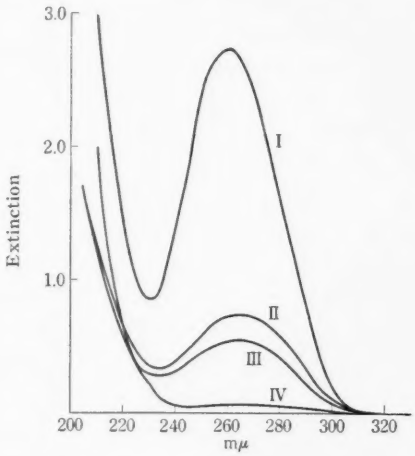


Fig. 5 Ultraviolet absorption curves of O-Fractions from rhodamine sarcoma, hepatoma, and NF sarcoma.

The absorption curves of TO-Fraction, original raw toxohrmone and its residue except TO-Fraction were compared in Fig. 6, in which was included the curve of

purified yeast nucleic acid as comparison in the same concentration. One can see from this table, that TO-Fraction almost lost the absorption peak at 265 mμ of the original sample, and that peak of the residue elevated in considerable degree. This table, therfore, shows that nucleic acid was not extracted by acid-



I Yeast RNA

II Raw Toxoharmone except TO-Fraction

III Original Raw Toxohormone

IV TO-Fraction

(The concentration of each sample was 0.1 mg/ml)

Fig. 6 Ultraviolet Absorption Curves of RNA, Raw Toxohormone, and TO-Fraction

methanol extraction, and preserved in the residue. It must be noted also that TO-Fraction showed no definite absorption peak at around 280 mμ.

In conclusion, from the ultraviolet absorption curves, it may be assumed that O and TO-Fractions do not contain nucleic acid in detectable amount.

Discussion

Because the O-Fractions from all kinds of tumors as far as tested showed definite catalase depressing activity, it is no doubt that they contain the same active principles as toxohormone, and there are some similarity between both fractions, for examples they are heat-noncoagulable and poor in tyrosine content. Nevertheless, it must be pointed out that there are also some differences between them, and the most remarkable one is that O-Fraction did not contain nucleic acid, and perhaps for this reason, it was not precipitable at two-fold volume alcohol addition at pH 4.5, but soluble in acid medium. It is will known that nucleoprotein can be made to precipitate by acid or alcohol, and this is the case with toxohormone. This fact may explain how it happened that Nakahara (2) and Endo (4) did achieve purification by the means of acid precipitation or dilute acid washing, in spite of the acid solubility of O-Fraction.

The second difference is the mode of action on thymus, that is, the effect of O-Fraction on this gland takes place at 24 hours, and that of the ordinary toxo-

hormone shows its maximm action at 48 hours after injection. We have no precise solution for this point, but from the results that the fractions I and II of the alcohol fractionation of O-Fraction exerted no catalase depressing activity but some thymus involuting one, we may suppose that there are different active principles for each action, and the one for thymus involution may be contained rather concentrated in O-Fraction. The histological changes in thymus, liver and other organs caused by the injections of O-Fraction were fairly remarkable, and will be published by some of our collaborators.

In addition to thymotropic action, O-Fraction exhibited antibiotic activity to E. coli at appreciable high dilution and the activity was concentrated only in fraction III of the alcohol fractionation. The antibiotic actions of basic proteins and/or peptides, for examples, histone (11) and protamine (12) have been widely reported. This action of O-Fraction is therefore not unexpected, considering its extraction procedure, that is, acid methanol extraction. And it was a clear difference between O-Fraction and ordinary toxohormone, the latter exerting no effect on the growth of E. coli. The details of the experiments of this line will be reported in any other paper.

Contrary to Endo's negative results about nucleic acid, recently Nakagawa et al. (9) obtained a tumor preparation active as toxohormone in so small a dose as 0.1 mg per mouse, which was called KNA by them and said to be composed of nucleic acid almost entirly. But they could not completely exclude the possibility of the contamination of some peptide in KNA. In our opinion, since O and TO-Fractions were extracted with acetic acid and highly soluble in acid medium, the nucleic acid fraction, that is KNA, extracted with hot TCA may contain protein or peptide of similar nature as TO-Fraction, and the protein moiety in KNA may have been responsible for the catalase depressing action.

In this connection, it is also recalled that almost all of the original activity in raw toxohormone was recovered in acid-methanol extraction, but the residue of that extraction yet retained catalase depressing activity comparable to the starting sample. Hitherto, we have done nothing with this residue, but since nucleic acid was reserved firmly in it without any loss, so we are now intending to isolate truely pure nucleic acid from it to make definite conclusion on the activity of nucleic acid.

Although the potency for catalase depressing action of O-Fraction became elevated over two-folds by ethyl alcohol fractionation, toxicity was also concentrated in the potent fraction. Then, for the purification of catalase depressing principle, we have to replace the starting material with TO-Fraction. The thymus involuting action of O-Fraction, however, was so remarkable, that it is hoped that it might offer the most convenient source for the purification of the active principle of this action.

In our endeavour for the purification of toxohormone fractions, we lately succeeded to concentrate it to the point where it is active in 2 mg doses, applying cellulose column chromatography and or benzoic acid absorption techniques on TO-Fraction. Details of this study will be reported later in another paper.

Summary

A fraction was obtained from acetone dry powder of rhodamine sarcoma, transplantable rat hepatome and NF-sarcoma with hot acetic acid-methanol (2 : 3) extraction. That from rhodamine sarcoma was effective in 20 mg injecting doses for mouse in depressing liver catalase activity and in reducing thymus weight, and those from hepatoma and NF-sarcoma were effective in 40 mg doses for both actions.

One sample of the fraction derived from rhodamine sarcoma was subjected to ethyl alcohol fractionation, and divided into 4 fractions. The activity for thymus involution was distributed almost equally among them, but catalase depressing action was demonstrated only in fraction No. 3, which was active in 10 mg dose.

The procedure of acid-methanol extraction was applied to raw toxohormone, and yielded a fraction effective in 10 mg doses for liver catalase depression, with no toxicity for mouse.

No nucleic acid contents were detected in any of these fractions by means of phosphate determination and ultraviolet absorption test.

Our hearty thanks are due to Dr. Nakahara for his kind interest and encouragement.

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要　旨

核酸をふくまないトキソホルモンの精製

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腫瘍のアセトン乾燥粉末を氷醋—メタノール (2：3) で加熱抽出し，トキソホルモン作用を呈する一分劃を得て O-Fraction と仮称した。ローダミン肉腫 (梅田) よりのものは，20 mg で，移植肝癌 (ラッテ)，NF 肉腫よりのものは 40 mg でマウスの肝カタラーゼを低下せしめ，さらに注射 24 時間後で胸腺を著明に萎縮せしめた。

ローダミン肉腫よりの一標本をアルコール分劃し 4 つの分劃を得た。各分劃はいずれも胸腺萎縮作用を示したが，肝カタラーゼ低下作用は第 3 の分劃にのみ認められ 10 mg で有効であった。

以上各腫瘍よりの O-Fraction は燐酸定量及び紫外部吸収試験で核酸をふくまないことが確認されたので，同じ抽出法を粗製トキソホルモン (アルコール沈澱の段階) に応用して同様に核酸を含まない 10 mg で有効な分劃を得た。このものは胸腺には注射 24 時間後では影響を示さなかった。

(文部省科学研究費による)

[GANN, Vol. 46; December, 1955]

FURTHER STUDIES ON THE GASTRIC LESIONS OF RATS BY ORAL ADMINISTRATION OF METHYLCHOLANTHRENE, WITH A CASE OF COLONIC ADENOCARCINOMA

(With Plates XXV—XXVIII)

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(Showa Medical School, Tokyo)

In the preceding paper (1), the induction of the hyperplastic lesions in the glandular stomach of rats receiving the emulsion containing 20-methylcholanthrene was reported. This communication presents the result of further studies on the production of gastric adenomas of rats and a case of an adenocarcinoma of the colon of a rat which received a greater amount of methylcholanthrene than in the case of previous studies.

EXPERIMENTS

Eighteen male albino rats of Wistar strain, 3 months of age and weighing 80–100 g were employed. They were fed on the rice diet with 2 per cent of sodium carbonate and were administered with the emulsion of the 20-methylcholanthrene by the similar method as previously reported. The method of the preparation of the emulsion has already been described, the emulsion being stabilized by the addition of a small amount of "Tween 80." The average daily intake of methylcholanthrene was about 1–5 mg per rat per day and it was larger than that in the previous studies. Food and fluid was not restricted, and fresh cabbage was supplied every two days.

Under the above experimental conditions several rats died during the first 50 days showing no significant change. On the 51 st day, one rat died with a lesion in the glandular stomach which proved to be an adenoma in nature upon microscopical examination (Fig. 1). All other rats died or were killed and autopsied after the periods ranging from 51 to 266 days during the oral administration of methylcholanthrene. Majority of them showed more or less definite adenomas or adenomatous lesions of the glandular stomach, as may be seen in Table 1.

Grossly, the gastric lesions manifested themselves usually as irregular and raised edges with a variable degree of excessive foldings. These conspicuous changes most frequently occurred in the distal portion of the pyloric region of the stomach. In the more advanced case (Rat No. 9), there was a firm, well-circumscribed area with a depressed, punched-out center 6 mm in diameter surrounded by an elevated ruffled border of mucosal thickening which shaded off

Table 1. Adenomas in the Glandular Stomach and Colonic Adenocarcinoma Induced by Oral Administration of Methylcholanthrene.

Rat No.	Period of Survival (days)	Gastric Lesions*	Colonic Lesions*
1	51	adenoma	—
2	55	adenoma	—
3	56	—	—
4	60	adenoma	—
5	64	—	—
6	65	adenoma	—
7	103	adenoma	—
8	152	—	adenocarcinoma
9	228	adenomatous diverticulum**	—
10	232	adenoma	—
11	266	adenoma	—
12	266	—	—

* All the diagnoses were confirmed by microscopical examination.

** Intestinalization of the gastric epithelium was observed.

gradually into the surrounding mucous membrane.

Microscopically, the lesions classified as adenomas varied in extent from small nodules of atypical acini in the mucosa and submucosa to large plaque-like glandular masses occupying the submucosa and invading the superficial muscularis. The glands composing the adenomas were irregular and distorted and lined by hyperchromatic, cuboidal, and columnar cells which varied in size, shape and staining. Very often the gastric adenomas were depressed below the surface, sometimes eroded, but usually not ulcerated; the margins were elevated and the muscularis mucosae were destroyed.

An adenomatous diverticulum occurred in a rat (Rat No. 9, died on the 228 th day). The lesion consists of atypical epithelial cells which extended to the serosa through the submucosa and the muscular coats (Fig. 6). There is chronic peritonitis with fibrous exudation. These appearances are similar to those observed by previous investigators (11). Intestinal epithelial cells were also observed in the areas adjacent to the diverticulum described above, and the pathological description of this lesion will be reported in detail in another publication.

All these gastric lesions seemed to have appeared earlier and severer than those in the previous experiments owing to the large amount of carcinogen administered at this time. However, the gastric lesions were not quantitatively related to the amount of carcinogen ingested or to experimental duration.

One rat (Rat No. 8) became moribund on 152nd day after the loss of weight, due to hemorrhages from tumors situated on the surface of the gastrointestinal tract. At autopsy, four masses or tumors of the colon and rectosigmoid were

found arising at 3, 8, 10 and 11 cm distal to the cecum, and measured 1.3, 1.2, 0.5 and 0.8 cm in diameter respectively. These tumors were firm, gray, nodular and often ulcerated on the mucosal surface. They were palpable as cancerous nodules on the peritoneum often covered with organizing granulation tissues. Tarry material was present, indicating old hemorrhage.

Microscopic examination of the colon revealed the adenocarcinoma infiltrating all coats of the colon and forming nodular masses on the serosal surface (Fig. 3). The cells of the tumor were arranged in small irregular acini and in solid nests and cords. Tumor cells were columnar, cuboidal, round, oval and deeply hyperchormatic (Figs. 5 and 8). Many mitotic figures were counted (Fig. 5). The stroma was composed of relatively avascular mature connective tissue and was more abundant in the areas of necrosis and inflammatory foci. On the mucosal surface of the tumor there were hyperplastic gland structures, and the mucous membrane on either side of the tumor showed atypical hyperplasia shading off into the adjacent mucosa (Fig. 7). There were no detached extensions of tumor tissue into the other tissues. No similar lesions were detected in other rats of the same group and it was felt that this was an induced tumor rather than a spontaneous tumor. The tumor has not been transplanted.

Discussion and Summary

In extensive experiments with mice and rats performed during the past decade, especially by Lorenz and Stewart and their co-workers (2–12), a relatively high proportion of precancerous and cancerous lesions were produced in the forestomach and small intestine by incoporating hydrocarbons in the diet, and gastric adenocarcinomas were readily produced by injecting methylcholanthrene into the wall of the stomach. No tumor, however, was induced in the glandular stomach and large intestine by oral administration of the carcinogenic substances. It was stated that the glandular stomach and large intestine are refractory to tumor formation because of the constant protective action of the mucous secretion.

On the other hand, several authors have reported on the occurrence of carcinoma of the colon in rats, using such substances as 2-acetylaminofluorene (13), radioactive yttrium (14), benzidine (15) and motor lubricating oil (16).

In this laboratory, it is demonstrated that the mucosae of the glandular stomach and large intestine in rat are not completely refractory to tumor induction. However, there is a strong contrast between the case of inducing tumors in the forestomach and small intestine (Lorenz and Stewart) and the production of adenoma or adenocarcinoma in the glandular stomach and colon (the present experiment).

The investigation on the production of hyperplastic lesions of the glandular stomach in rats by oral administration of methylcholanthrene were extended by

an increase in amount of carcinogen ingested. Majority of rats showed more or less definite adenomas or adenomatous lesions in the glandular stomach. Intestinal epithelial cells were observed in the gastric mucous membrane of one rat. An colonic adenocarcinoma with mucosal hyperplasia was induced in one out of 12 rats.

The authors are indebted to Mr. S. Noda for the microphotographs.

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要　旨

メチルコランスレン経口投与による白鼠の腺胃腺腫並びに結腸腺癌の生成について

森　和雄，一井昭五，重田吉輝
(昭和医科大学医動物学教室)

メチルコランスレンを乳剤として白鼠に経口的に与え，その腺胃部に腺腫様過形成が生成できることは前報で示した通りである。本報ではメチルコランスレンをやや大量に動物に与えることによって前回より確実にかつ短期間に胃腺腫が生成されることを記載した。就中1例では胃粘膜の腸上皮化生を伴う腺腫様変化がみとめられた。さらに他の1例では結腸に腺癌の形成がみとめられた。

(文部省科学研究費による)

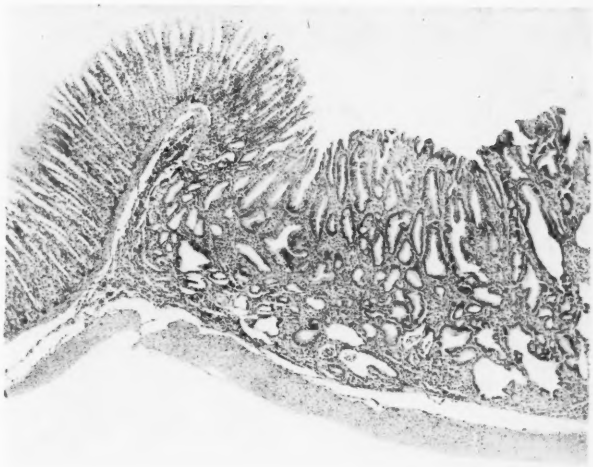

Fig. 1. Rat No. 1 died on 51st day. Adenoma of the glandular portion of the stomach.

Fig. 2. Rat No. 4 died on 60th day. Adenoma of the glandular portion of the stomach. Transition zone of the normal mucosa is seen on the left half.

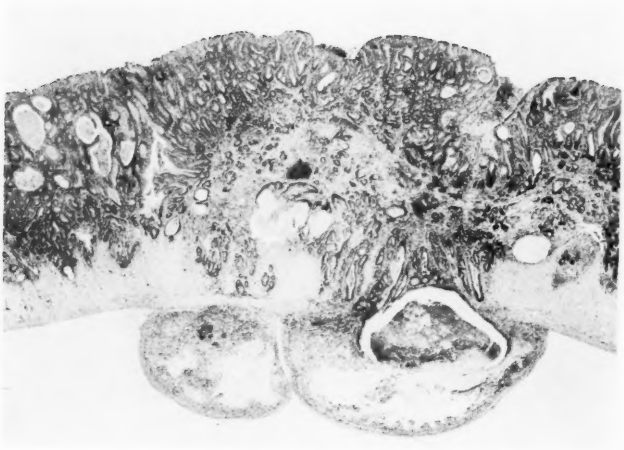

Fig. 3. Rat No. 8 died on 152nd day. Section through adenocarcinoma of the ascending colon. There are invasion and destruction of the muscularis, formation of the tumor nodules on the peritoneal surface.

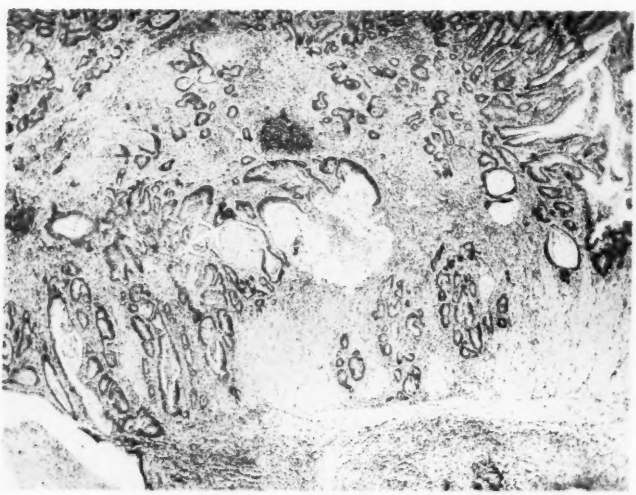

Fig. 4. High power view of the area shown in Fig. 3. Note the neoplastic infiltration into the muscularis propria.

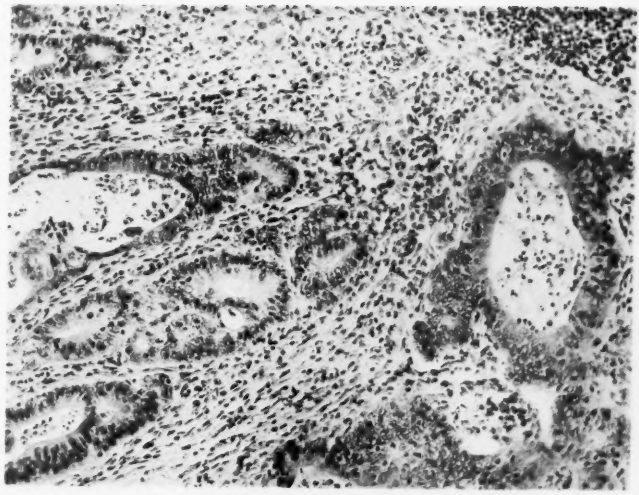

Fig. 5. High power view of the area shown in Fig. 4.

Fig. 6. Rat No. 9 died on 228th day. Antrum (duodenum on right). Adenomatous diverticulum. Intestinal epithelial cells are shown in the area adjacent to the diverticulum.

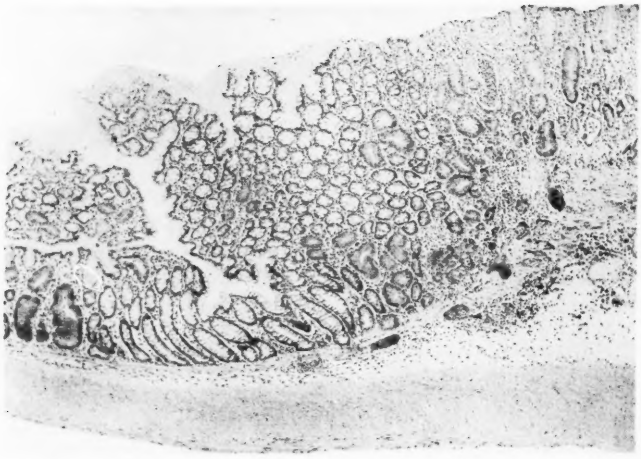

Fig. 7. Rat No. 8 died on 152nd day. Section through adenocarcinoma of the descending colon. Transition zone of the normal mucosa and the adenocarcinoma.

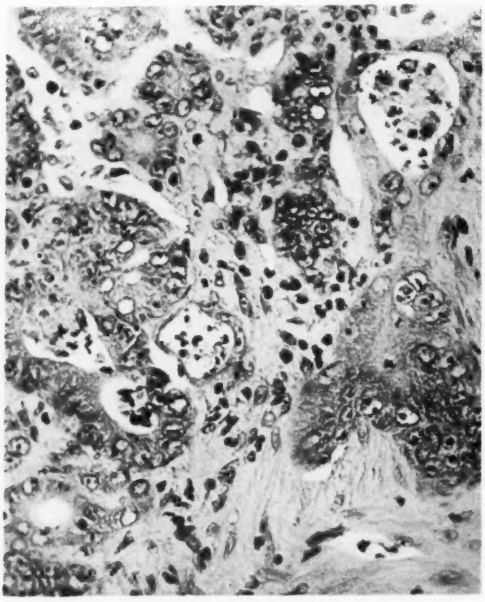

Fig. 8. High power view of the area shown in Fig. 7.

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